Foraging behaviour of the ruffe (*Gymnocephalus cernuus*) and predator avoidance by the freshwater Isopod *Asellus aquaticus*: Implications for predator-prey interactions.

by

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Summary

This thesis describes a study of aspects of the foraging behaviour of ruffe (*Gymnocephalus cernuus*), anti-predator responses in an important prey species (*Asellus aquaticus*), and the interactions between them.

A pilot study was conducted to examine use of shelter by small groups of *A. aquaticus*. This showed that *A. aquaticus* aggregated in shelter, and formed the basis for later experimental design (chapter 2).

To examine the trade-off between use of shelter and feeding, 53 groups of 10 individually marked *A. aquaticus* were observed over 1 day in tanks containing food and sterile, food free shelter (in the form of artificial plants), separated by a brightly lit section of bare sand. The proportion of time spent foraging and in shelter was compared in different reproductive categories of *A. aquaticus*. Brooding females spent more time in shelter than other groups (chapter 2). Behavioural changes within the brooding cycle were examined; the trade-off that females made between seeking shelter and food was influenced by stage in this cycle, with females in early stages spending more time in shelter and females at the end spending more time feeding (chapter 2).

A similar experimental set up was used to examine possible effects of chemical cues from a potential predator on shelter seeking and foraging in *A. aquaticus*.

Shelter use and activity of *A. aquaticus* was quantified on 2 successive days, one with 'clean' water straight from Loch Lomond pumped through the tanks, and the second with water pumped from a fish tank containing several brown trout (*Salmo trutta*). This experimental protocol was used, because only one tank was available in which to carry out these experiments. A control was conducted with 'clean' water provided on both of the 2 days. In males use of shelter was greater when predator cues were introduced than they had been prior to introduction. However this could not be clearly ascribed to the cues themselves, since shelter use also increased (albeit less strongly) in a control study, where water was added on a second day. Possible improvements to experimental design are discussed (chapter 3).

Previous studies of ruffe have indicated that the lateral line is important in foraging. The behaviour of ruffe was therefore studied while foraging for live concealed prey in the dark. Individual fish were trained over several weeks to forage in the dark for a few hours a day, in an arena with an array of pots, some of which contained live bloodworm buried in gravel and others which contained gravel alone. Foraging was recorded using a video camera under infrared light, and the tapes analysed to show general foraging responses and the frequency and duration of foraging visits to all pots during sessions lasting two hours. Seven sessions were recorded in this way, and the first 2 hours of foraging analysed in detail. In general from early on in the foraging sessions ruffe made more frequent visits to pots with food; this is interpreted as evidence that they can use non-visual cues to detect concealed prey. Foraging behaviour of some fish indicated that they learn the location of profitable food patches during each session (chapter 4).

To examine anti-predator responses of *A. aquaticus* and their effects on foraging attempts by ruffe, predatory behaviour of ruffe towards *A. aquaticus* were observed in the presence and absence of shelter, in well lit tanks. Use of shelter by *A. aquaticus* conferred strong protection against ruffe. In the absence of shelter, different predator avoidance tactics were used, based largely on immobility, exploiting the dependence of ruffe on prey movements. Selection of prey by ruffe was examined, and in these feeding conditions no preferences according to size and gender were found (chapter 5).

A sample of ruffe from Loch Lomond was examined for morphological status and the presence of macroparasties. Age was determined using a relatively new technique based on rings in the dorsal fin rays. Ages ranged from 0 to 6 years and sizes from 5 to 10 cm. Females were larger and heavier than males, had slightly larger gonadosomatic indices, but did not differ from males in body condition. A small proportion of fish were infected with the nematode *Camallanus lacustris*, but most of them had *Diplostomum* infection in one or both eyes; in some cases more than a hundred flukes were found per fish. Intensity of *Diplostomum* infection was similar in males and females, and increased significantly with fish age. No adverse effects on condition were observed, even in fish with very heavy infections. These findings can be related to the diet and foraging behaviour of ruffe (chapter 6).

The results described in this thesis are discussed in relation to existing literature on foraging behaviour in ruffe and their status as a newly introduced species in Loch

Lomond. Possible improvements to the design of this study are discussed and future areas for further research identified (chapter 7).

Chapter 1

General introduction

1.1 Ruffe (*Gymnocephalus cernuus*) as an introduced species

Ruffe are of great interest to biologists due to their success as an invading species, across various locations in both Europe and North America (Kålås, 1995; Maitland *et al.*, 1983; Pratt *et al.*, 1992.) Their success in colonising new areas indicates that they are well adapted to living in a variety of habitats. Because of this, the biology and ecology of ruffe is a growing area of research, and further understanding of this species may give clues as to their impact on indigenous species following introduction.

Invasions by ruffe have occurred in other areas in Europe, including Lake Mildevatn, Norway (Kålås, 1995). There is much concern regarding the introduction of ruffe to Lake Superior. First recorded in 1987, the ruffe population has rapidly increased. Introduction is thought to be a result of the release of ballast water from large cargo ships travelling from Europe (Pratt *et al.*, 1992). Lake Superior has large numbers of economically important fish species, and there has been growing concern that ruffe may be competing with the indigenous yellow perch (*Perca flavescens*) and trout-perch (*Percopsis omiscomaycus*) for benthic invertebrates (Ogle *et al.*, 1995). Ruffe are also thought to threaten other fish species, such as the lake herring (*Coregonus artedii*), through predation on their eggs (Selgeby, 1998).

Following the introduction of ruffe to Loch Lomond, which was first recorded in 1982 (Maitland *et al.*, 1983), the population has rapidly expanded and spread to the extent that they are now one of the most commonly occurring fish species in the loch

(Adams, 1994). The nature of the introduction of ruffe to Loch Lomond is unclear, but it is thought that pike anglers using ruffe as live bait are responsible for their introduction (Maitland *et al.*, 1983).

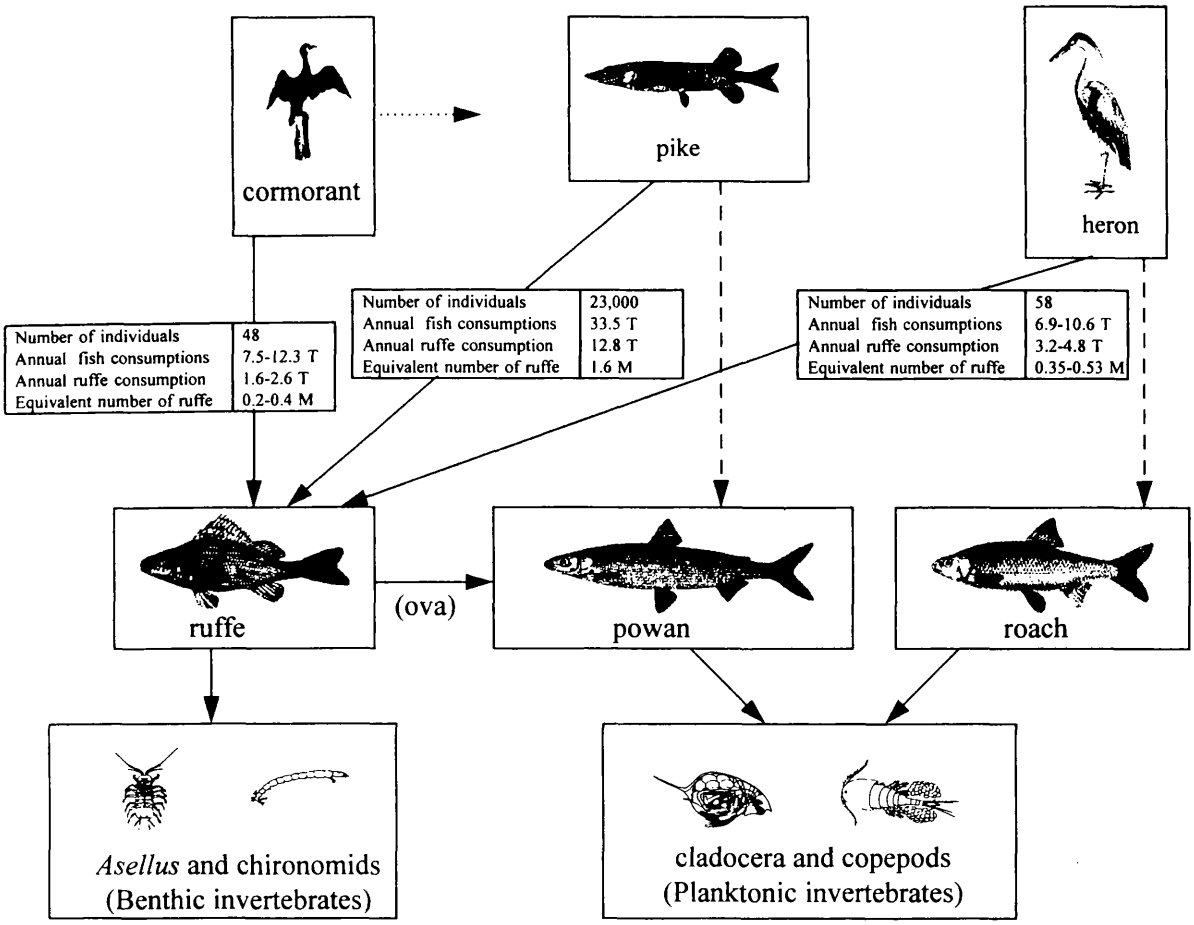
Following the introduction of ruffe, numerous changes have occurred in the population structure and food web in Loch Lomond (Adams and Maitland, 1998) (see figure 1.1 for diagram of the changes to the food web in Loch Lomond). Since the ruffe invasion, the diets of some top predators in the Loch changed markedly. Pike (*Esox lucius*) that fed primarily on powan (*Coregonus lavaretus*) prior to the ruffe introduction, now feed largely on ruffe (Adams, 1991), possibly due to its relatively poor swimming ability. A dietary shift has also occurred in two piscivorous birds, with cormorants and herons now feeding primarily on ruffe (Adams, 1994). In addition to the effect on community structure of Loch Lomond by the invasion of an additional prey species, introduction of ruffe may have a disruptive effect through its predation on indigenous species. For example, ruffe are known to feed on the eggs of powan (*Coregonus lavaretus*), which is an endangered fish species in Loch Lomond (Adams and Tippett, 1991).

1.2 The ruffe diet

The diet of ruffe in Loch Lomond has been studied extensively, in order to gain more information as to the relationships it has with indigenous species. It was of particular interest to document the diet of ruffe in Loch Lomond, in order to ascertain whether

Figure 1.1

Food webs in Loch Lomond before and after the introduction of ruffe (From Adams and Maitland, 1998)



Boxes show:

Estimated population of predator

Estimated fish consumption (tonnes)

Estimated ruffe consumption (tonnes)

----------▶ Main prey item before ruffe became established

——————▶ Main prey item since ruffe became established

ruffe were competing with perch for food; or whether they were affecting the population of powan through egg predation.

The diet of ruffe in Lake Superior (North America) has also been studied, (Ogle *et al.*, 1995). Ruffe were found to feed largely on benthos; however there were differences in prey preferences between age 0 individuals and adults. During early summer, age 0+ ruffe fed mainly on cladocerans and copepods, but chironomids became the main component of the diet during late summer and autumn. There were also differences in the diet of adults which was dependant on size; most fed mainly on chironomids and microcrustaceans, but adults larger than 12cm had a more varied diet, feeding mainly on chironomids, burrowing mayflies and caddisflies.

The foraging behaviour of the different age classes also differed, with age 0+ remaining in deeper water, feeding throughout the day, whereas adults migrated at night to feed in shallower water. The foraging behaviour of ruffe in Lake Superior is very similar to that of ruffe in Lake Aydat (France), where they are nocturnal benthivores (Jamet and Lair, 1991). The foraging activity of ruffe in Loch Lomond has not been studied directly, although their diet has been well documented. Ruffe in Loch Lomond predominantly feed on benthic invertebrates. Although they have been caught in gillnets within the littoral zone, this is thought to be due to the seasonal migration into shallower water for spawning. The diet of ruffe in Loch Lomond varies between the different basins and between seasons (Adams and Maitland, 1998). In summer ruffe in the south basin predominantly feed on Diptera larvae (73.1%) consisting mainly of chironomids (larvae of the non-biting midge), whereas in the

mid-basin at the same ruffe feed mainly on *A. aquaticus* (46.3%), whilst still taking relatively large number of chironomids (29.3%). The diet of ruffe during the winter is quite different, with ruffe consuming large numbers of fish eggs (39.4%) in addition to *A. aquaticus* (30.8%) (Adams and Maitland, 1998). In a review of published data on ruffe diets, many similarities were seen in the major prey species eaten by ruffe in different lakes (Kålås, 1995). Chironomids, *Gammarus* sp., *Asellus*, and *Pallaesea* were all dominant prey items in these studies. Examination of the ruffe diet and activity in Lake Mildevatn (Norway), was very different to that found in other studies (Kålås, 1995). In Lake Mildevatn ruffe were mainly active during the day, not at night, as suggested by other studies. Ruffe mainly fed on zoobenthos during the winter (October to March). From April this gradually changed until a predominance of zooplankton, including *Daphnia* sp. and copepods were found in ruffe stomachs during June to September. Ruffe were also found in deeper water during the winter, when compared with the summer (June to October), possibly due to spawning in shallower water during the early summer.

1.3 Foraging behaviour and prey detection by ruffe

Ruffe have been of interest to biologists for some time, due to their well developed lateral-line system, which is usually limited to deep sea fish, and is not seen in other Percids. Several studies have examined the morphology and activity of the lateral line (Gray and Best, 1989; Jackubowski, 1963, Wubbels, 1991; Wubbels *et al.*, 1990) and its development, (Disler and Smirnov, 1977), several of these studies have suggested that the lateral line of ruffe is important in foraging. Disler and Smirnv (1977)

examined the development of the lateral-line canals in both ruffe and perch (*Perca fluviatilis*), relating this to the role the lateral line plays in foraging in ruffe, and suggesting it may also be important when detecting the presence of large predators. They described ruffe as a "...solitary, secretive fish which feeds on or near the bottom in low light conditions on midge larvae...." (Disler and Smirnov, 1977). Studies on the nerve activity within the large sensory canals located in the head, have shown them to be highly sensitive to background vibrations (Gray and Best, 1989; Wubbels *et al.*, 1990), and capable of locating the source of a stimulus by these senses alone (Wubbels, 1990). Studies on the foraging behaviour of ruffe have been conducted mainly from a perspective of them as competitors of perch. It has been demonstrated that perch are better swimmers and more effective foragers than ruffe (Bergman, 1987; Bergman, 1988). Other studies have commented on the poor swimming ability of ruffe, which may increase their risk of predation (Adams, 1991). When the foraging behaviour of ruffe and perch on free swimming invertebrate prey (*Chaeoborus obscuripes*) was examined under a variety of different environmental conditions, it was found that ruffe are less sensitive to low temperatures than perch. Perch displayed reduced swimming and foraging abilities as temperatures below 8°C, whereas the performance of ruffe was not influenced by temperature (Bergman, 1987). In a different study Bergman (1988) examined the influence of different light intensities on the foraging abilities of both ruffe and perch on *Daphnia magna* and *Chaeoborus obscuripes*. Light intensity effected the foraging abilities of both fish, which decreased as light intensity decreased. However, perch were more successful than ruffe when foraging at high light intensities, whereas ruffe had higher success rates than perch when in total darkness (Bergman, 1988). Both these studies indicate

that ruffe are well adapted to foraging under fluctuating environmental conditions, and are able to effectively use non-visual senses in locating and capturing prey. This suggests that although ruffe may be out-competed by perch under optimal conditions, ruffe are able to occupy habitats that perch are excluded from because of light and temperature constraints. However, these experiments were carried out using prey common to perch and not to ruffe. In Loch Lomond Cladocera constitute a very small proportion of the ruffe diet, whereas in perch most of the diet consists of Cladocera (Adams and Maitland, 1998). Ruffe typically forage on benthic invertebrates, and in Bergman's studies the foraging abilities of perch and ruffe were only examined when foraging on pelagic prey species. Two more recent studies, investigating the foraging behaviour of ruffe and yellow perch (*Perca flavescens*), have used benthic prey (Janssen, 1997; Savino and Kolar, 1996). When the foraging behaviour of both these fish was studied while foraging for chironomids (a benthic prey species common to both fish), ruffe appeared to be more efficient foragers than yellow perch (Savino and Kolar, 1996). Janssen (1997) studied the foraging behaviour of ruffe and yellow perch under conditions of darkness, when foraging on *Daphnia magna* (pelagic prey) and *Hexagenia limbata* (benthic prey). Ruffe proved to be a more effective predator than yellow perch, on both prey species. Yellow perch were very limited when feeding on *D. magna*, but successfully fed on *H. limbata*. This suggests that yellow perch do employ their lateral line when foraging in the dark, although as the comparative anatomy of the lateral lines of both species suggests, ruffe have a more sensitive lateral line than yellow perch. When the effects of habitat complexity were studied, foraging efficiency of both perch and ruffe on *A. aquaticus* and *Corophium volutatōr* decreased with increasing complexity, (Mattila, 1992).

These studies highlight the importance of fluctuating environmental conditions on influencing the foraging abilities of ruffe. In this thesis I was interested in how ruffe would overcome sub-optimal foraging conditions, and whether different foraging strategies were used when foraging under different conditions for different prey species; using *A. aquaticus* and chironomid larvae as prey, which are both important in the diet of ruffe in Loch Lomond.

1.4 Biology of *Asellus aquaticus* and closely related taxa

Both isopods and amphipods are important prey species in the diets of numerous aquatic predators, especially fish (Brattey, 1983; Giles and Tippett, 1987; Holomuzki and Short, 1988; Holomuzki and Short, 1990; Huang and Sih, 1991; Mathis and Hoback, 1997; Rask and Hiisivuori, 1985). *Asellus aquaticus* was particularly important to the present study, due to its importance in the ruffe diet (Kålås, 1995; Mattila, 1992), especially in Loch Lomond (Adams, 1994; Adams and Maitland, 1998).

A. aquaticus is a freshwater isopod, which is found widely distributed across a variety of freshwater habitats. *A. aquaticus* is a benthic macroinvertebrate, which feeds predominantly on detritus, fungi and algae, including periphyton which they also use as shelter (Cummings and Klug, 1979; Graça *et al.*, 1994a; Graça *et al.*, 1994b; Panov *et al.*, 1995; Rask and Hiisivuori, 1985; Rossi and Fano, 1979). *A. aquaticus* are also cannibalistic (personal observation), feeding on both live and dead conspecifics. I am not aware of this being documented in previous publications in this particular species,

but there are other documented examples of cannibalistic isopod and amphipod species. A study on the amphipods *Gammarus pulex* and *Gammarus duebeni* found that cannibalism occurred especially frequently on newly moulted individuals (Dick, 1995). Large individuals of the isopod species *Saduria entomon* cannibalise smaller individuals, (Sparrevik and Leonardsson, 1995). The isopod *Thermosphaeroma thermophilium*, is a highly cannibalistic species (Shuster, 1981). Cannibalism in this species influences microhabitat selection by different genders, females select areas in order to avoid males, which are cannibalistic on them, and juveniles avoid both males and females to reduce the risk of cannibalism (Jormalainen and Shuster, 1997). The study found that juveniles were found on vegetation more often than adults of either gender, whilst females were more abundant on vegetation than on the substrate, where males most frequently occurred.

Reproduction in peracarid crustaceans

Mate-guarding is a common feature of reproduction in many different species. Males in both amphipod and isopod species mate-guard females prior to mating (precopulatory mate-guarding). This strategy has evolved due to the short time that females are available for fertilisation, (amphipods: Dunn, 1998; Dunham *et al.*, 1986; Dunham and Hurshman, 1990; Elwood and Dick, 1990; Hatcher and Dunn, 1997. isopods: Ridley and Thompson, 1979; Sparkes *et al.*, 1996; Shuster, 1981. review: Jormalainen, 1998). This enables males to ensure that they have access to females in the short period following moulting, in which they are receptive. In *A. aquaticus* females can only be fertilised up to 24 hours after moulting (Ridley and Thompson, 1979; reviewed in Jormalainen, 1998). Isopods like *A. aquaticus* undergo a two-stage

moult, where the posterior end moults first followed by the anterior. Females can only be fertilised in the period between the posterior and anterior moult (Ridley and Thompson, 1979). During precopula, the male carries a single female underneath him until she is ready to fertilise; in *A. aquaticus* this may last for several days (up to 8 days). Following fertilisation, females carry their developing young in a specialised brood pouch (marsupium) on the ventral surface of their thorax for several weeks (depending on the species) before the young leave their mother, once they can fend for themselves.

Throughout mate-guarding competition occurs between males for females, with other males attempting to 'take over' the female, by trying to push the other male off (Ridley and Thompson, 1979). In the stock tanks it was not uncommon to see numerous males clumped together around just one mate-guarding pair. In isopod and amphipod species, this mate selection is not random, males select females on a basis of size and proximity to moulting (Adams *et al.*, 1985; Ridley and Thompson, 1979). It is less costly to a male to mate-guard for a shorter time, partly because of the costs incurred by carrying a female, but also because they must avoid take-over attempts by competing males.

The mechanism by which males assess female suitability for mating is not fully understood. It has been suggested that male *A. aquaticus* use a form of chemoreception to gain information about the proximity of females to a sexual moult, possibly by assessing levels of the moulting hormone crustecdysone (Ridley and Thompson, 1979).

Size-assortative pairing has been observed in many different amphipod and isopod species, where males preferentially guard larger females (amphipods: Elwood and Dick, 1990; Hatcher and Dunn, 1997; isopods: Adams *et al.*, 1985; Ridley and Thompson, 1979; Shuster, 1981; review: Jormalainen, 1998). The advantage for males of doing this is clear, because larger females produce larger broods. There may however be associated costs of guarding larger females, because males must carry females during guarding, larger females may make manoeuvrability more difficult (Adams *et al.*, 1985; Elwood and Dick, 1990), which may have implications for increased predation risk and increase the energetic costs of guarding. Larger females may also be more difficult to defend, and may attract more competition between males. There are a variety of ways that males overcome this. Hatcher and Dunn (1997) found that *Gammarus duebeni* males selected larger females up to a threshold size, and that particularly large females were less likely to find mates, due to the difficulty associated with finding males large enough to guard them. Adams *et al.* (1985), found that male *A. aquaticus* carrying larger females were less mobile, walking more slowly that when they guarded smaller females. This suggests that there are costs associated with maximising reproductive output by mating with larger females.

The majority of previous studies have concentrated on the costs of reproduction in amphipods and isopods from the perspective of costs to males, associated with mate-guarding, (Sparkes *et al.*, 1996). In our study we were interested in not only the influence of mate-guarding on shelter seeking and predation risk, but also the

influence that brood carrying had on females. Previous studies have largely neglected brooding females, although a reduction in the manoeuvrability of females *A. aquaticus* during brooding has been noted (Lyndon, 1996). A recent study on the amphipod *Crangonyx pseudogracilis* examined the maternal behaviour of brooding females (Dick *et al.*, 1998). They found that females of this species actively care for their broods, circulating water through them to increase ventilation, and removing nonviable eggs from the brood pouch. The intensity of this brood care changed throughout according to the developmental stage of the brood, demonstrating that females are sensitive to development in their young. Whilst carrying out these experiments, I was interested in examining the influence of various different reproductive states on foraging, shelter seeking (chapter 2) and predation risk (chapter 5) in *A. aquaticus*, with the intention of relating any findings to the predator-prey interactions between ruffe and *A. aquaticus*.

1.5 Anti-predator strategies, including foraging and predation trade-offs

Prey species face a variety of decisions, associated with balancing the demands of activities such as of foraging and reproduction, with avoiding predation (reviewed in Lima and Dill, 1990). There are a variety of different ways in which prey can reduce the risk of predation, and assess the level of risk that they are subjected to.

Predator avoidance strategies

A common strategy employed by animals in avoiding predation, is to make themselves less conspicuous to predators. Cryptic colouration, when individuals are

able to be camouflaged against the background can be an effective anti-predator strategy when avoiding predation by visually mediated foragers (Ugolini, 1997). The effectiveness of cryptic colouration is dependant on individuals remaining against suitable backgrounds. If an individuals needs to move between microhabitats with different backgrounds, fixed colouration may result in them becoming more conspicuous to predators (Merilaita *et al.*, 1999). In these cases the ability to use other methods of predator avoidance will be important.

One method of doing this can be by reducing activity, which can be an effective strategy against both visual predators, and those which use mechanosensory cues in foraging. Dragonfly larvae (*Tetragoneuria cynosura* and *Ladona deplanata*) both increase shelter use and decrease foraging in the presence of predatory fish (bluegill, *Lepomis macrochirus*) (Pierce, 1988). Many species use this as a strategy for reducing predation risk, but this can be costly in terms of lost foraging opportunities and may incur a fitness cost. This can be seen in the isopod *Lirceus fontinalis*, which reacts to the presence of predatory green sunfish (*Lepomis cyanellus*) by both reducing activity and remaining in macroalgal (*Cladophora*) shelters. These shelters are not rich in food resources and when exposure to predacious fish is prolonged, a reduction in growth rate can be seen (Holomuzki and Short, 1988). In isopods, the number of eggs produced by females is dependent on body size, and therefore prolonged use of unprofitable feeding patches due to the presence of predators will lead to a reduction in fecundity. Implications for males can also be great, as a large body size in isopods is essential for mate-guarding larger, more fecund females and also in competing with other males for mates (Ridley and Thompson, 1979; Shuster,

1981). *L. fontinalis* displayed the same behavioural adaptations in the presence of chemical cues from green sunfish, even though no predators were present. This suggests that these isopods can assess the risk of emerging from shelters without having to put themselves at risk, (Holomuzki and Short, 1988).

Morphological adaptations can also reduce the risk of predation by making prey consumption either more difficult or unpleasant. When snails (*Physa gyrina*) were exposed to chemical cues from injured conspecifics (*Orconectes rusticus*), their behavioural responses differed according to body size (Dewitt *et al.*, 1999). Small individuals displayed high levels of antipredator behaviour, by moving out of the water, away from potential aquatic predators, whereas larger snails did not show significant anti-predator behaviour. This difference in behavioural responses to predation risk, correlates to the risk experienced by snails of different sizes in the wild. Crayfish are limited to foraging on smaller snails, as larger snails are more difficult to crush. Therefore small snails appear to be compensating for their lack of morphological defences to predatory crayfish, by adapting their behaviour (Dewitt *et al.*, 1999).

Use of olfactory cues in avoiding predation

Any adaptation that allows prey species to pre-empt a predatory strike, by gaining information regarding predation risk early on, is advantageous. This can be especially true in animals where visibility is reduced, either by dense vegetation (Mattila, 1992), darkness (Metcalfe *et al.*, 1997), or under conditions of high turbidity (in aquatic habitats only) (Abrahams and Kattenfeld, 1997). The aquatic snail (*Physa gyrina*)

(see above, review of reference Dewitt, *et al.*, 1999), can detect the presence of injured conspecifics from chemical cues, and adapt their behaviour accordingly. *Gammarus pulex* is able to detect chemical cues from predatory fish that are downstream, by both utilising and creating backflows in the water current (Dahl *et al.*, 1998). This was demonstrated when *G. pulex* actively avoided trout and trout odours that were present downstream. Cues from predator diets can also be important in decisions regarding the use of anti-predator behaviour. Malmqvist (1992) examined the responses of several freshwater invertebrates to chemical cues from a variety of different predators. Anti-predator responses were exhibited in many of the species tested, with the largest reduction in activity occurring in *Baetis rhodani* (a mayfly nymph) and *Agapetus ochripes* (a caddisfly larvae) in response to cues from *Dinocras cephalotes* (a large predatory stonefly nymph).

Reproduction as a factor influencing predation risk

Reproduction can increase the risk of predation in both males and females (reviewed in Magnhagen, 1991), and therefore many species adapt their behaviour in order to reduce the potential risks. Males of many species have evolved elaborate courtship displays through female sexual selection, (Magurran and Seghers, 1990). These may increase the mating potential of males, but can also attract predators as well as females, increasing the costs of reproduction for males. Even in species where no elaborate attention-seeking displays are involved in gaining a mate, males can suffer elevated levels of predation. Males often spend much of their time during the breeding season searching for mates. This increased mobility increases the chance of

coming into contact with predators (Gwynne, 1987), and can also increase conspicuousness of males to visually foraging predators.

Animals that involve a period of mate-guarding in the mating process can also put themselves at heightened risk of predation, as it can make them more conspicuous to predators, and less able to escape (Rowe, 1994). Some species reduce this risk by modifying their behaviour by reducing their activity, or hiding in shelter during these stages. In the isopod *Lirceus fontinalis*, males guard females prior to mating, pairs in precopoula were found most often concealed under stones (Sparkes *et al.*, 1996). It is thought that use of refuges reduces the cost of guarding for males, because carrying females in precopula is energetically costly, and remaining in shelter can conserve energy, but also it reduces their chances of being subject to competition from other males, and decreases predation risk.

Increased predation risk is also a cost associated with reproduction in females. In species that carry their young during and after development locomotion may become reduced, which can reduce the chances of escaping predation when a predator is encountered. This phenomenon is seen in a variety of species. (Cooper *et al.*, 1990). Many females reduce the predation costs associated with reproducing by modifying their behaviour. Female grass shrimps (*Palaemon adspersus* and *Palaemon elegans*) which carry their eggs underneath their abdomen during breeding, have reduced escape speeds when compared to non-ovigerous females (Isaksson, 1999). Reduced agility has also been noted in *A. aquaticus* carrying broods, (Lyndon, 1996). Carrying eggs can also increase the risk of predation by visual foragers, in cases where eggs make females more conspicuous. Pumpkinseed fish (*Lepomis gibbosus*) and yellow

perch (*Perca flavescens*) fed selectively on female *Daphnia* sp. that were carrying eggs. The eggs of *Daphnia* sp. are pigmented, and increase the visibility of individuals to visual predators, such as these predatory fish (Mellors, 1975).

1.6 Influence of parasitic infection on predator-prey interactions

Parasites are known to influence predator and prey interactions in a variety of ways. They can do this by either effecting the prey species, making them more vulnerable to predation (reviewed by Moore and Gotelli, 1990), or by effecting the ability of a predator to forage.

Influence of parasitism on predators

The eye fluke *Diplostomum* spp. (Digenea) is found in the eyes of many different fish species (Kennedy, 1974). *Diplostomum* infections have been shown to adversely effect the sight of fish, even when infections are relatively light, (Owen *et al.*, 1993). Because of the negative effect that *Diplostomum* has on fish sight, it has been linked with reduced foraging ability in a variety of visually foraging fish. When the behaviour of dace (*Leuciscus leuciscus*) with *Diplostomum spathaecum* infections was studied, it was found that because of their reduced foraging ability as a result of reduced visual acuity, they modified their feeding behaviour, spending more time foraging, and foraging closer to the water surface (Crowden and Broom, 1980). This may put them at direct risk from piscivorous birds, which are the definitive host of this parasite. This is an example of how a parasite that alters the behaviour of its intermediate host (whether intentionally or simply as a by-product of infection),

increases the chance of being passed on to its definitive host for complete development.

Influence of parasitism on prey

The trematode *Microphallus* sp. is an example of a parasite that alters the behaviour of its intermediate host, the aquatic snail *Potamopyrgus antipodarum*, making it more vulnerable to predation by waterfowl, the definitive host of this parasite species (Levri, 1998). The change in behaviour that *Microphallus* induces in *P. antipodarum* is not simply an incidental product of infection, but is more specific (Levri, 1999). Infection by *Microphallus* increased the amount of time spent feeding by snails, which may be due to an increase in energetic demands as a result of infection. Infected snails also spent more time exposed on the top of rocks during the day, even in the absence of food. This indicates a more specific alteration in host behaviour by the parasite, as this increases the chances of infected snails being consumed by predacious waterfowl (Levri, 1999).

Isopods and amphipods are infected by a variety of acanthocephalan worm species. Acanthocephalans are spiny headed worms, that as adults live in the intestines of a numerous of fish species, including ruffe (Kennedy, 1974). They are known for their ability to alter the appearance and behaviour of their isopod (Oetinger and Nickol, 1981) and amphipod intermediate hosts, increasing their risk of predation. A study of the influence of a variety of acanthocephalan parasites on the behaviour of amphipods to light and disturbance found that infected individuals responded differently to

uninfected conspecifics, although the nature of this behavioural modification was specific to different parasite and host species (Bethel and Holmes, 1973).

Isopods infected with acanthocephalans often experience a change in pigmentation in addition to a change in behaviour. *Acanthocephalus lucii* infection in *Asellus aquaticus* causes the melanization of the respiratory operculae (Brattey, 1983). The definitive hosts of *A. lucii*, are a variety of freshwater fish, most noticeably the percids, (including ruffe). Visibly darker operculae may cause *A. aquaticus* to become more conspicuous to visually foraging predators such as the perch, facilitating parasite transmission to definitive host species. When the effect of parasitism on predation was studied, perch selectively fed on *A. lucii* infected *A. aquaticus*, eating significantly more infected than uninfected individuals (Brattey, 1983). This could have been due to increased conspicuousness or because infected individuals suffer a reduction in agility, and may therefore find it more difficult to escape predation (Lyndon, 1996).

A more recent study investigated whether *A. lucii* and *Acanthocephalus anguillae* infection altered the behavioural responses of *A. aquaticus* to light, disturbance and heat (Lyndon, 1996). *A. anguillae* have a more marked influence on the colour of *A. aquaticus*, that *A. lucii*, causing melanization of the whole body, (Dezfuli *et al.*, 1994). *A. lucii* infection did not alter the behavioural responses of *A. aquaticus*, but *A. anguillae* infected individuals experience a variety of changes in their behaviour (Lyndon, 1996). These individuals were attracted to both light and disturbance, which

could result in an increased risk of predation from definitive hosts of *A. anguillae*, such as the barbel (*Barbus barbus*).

Studies on other acanthocephalan infections of isopods have shown behavioural changes more specific to increased predation risk. *Acanthocephalus dirus* infection in the isopod *Caecidotea intermedius* modifies anti-predator behaviour (Hechtel *et al.*, 1993). Uninfected individuals actively avoid creek chub (*Semotilus atromaculatus*), a predator of isopods and definitive host of *A. dirus*, seeking out shelter. Whereas infected conspecifics, which are both lighter in colour and more active, displayed an attraction to the fish predators, and spent more time in open water, seeking cover less frequently.

Previous unpublished surveys of the parasite fauna of ruffe in Loch Lomond had found the parasite *Acanthoceohlus lucii* (an acanthocephalan worm) in the intestines of ruffe (pers. com. D.W.T Crompton). During this study, I had hoped to examine the influence of *A. lucii* infection in *A. aquaticus*, in relation to shelter use and foraging. This was not possible, as after extensive invertebrate surveys of many different field sites, I was unable to find any infected individuals. A survey of macroparasites in ruffe from Loch Lomond also failed to find any acanthocephalan species.

1.7 Aims

The overarching aim of the work described in this thesis is to contribute to understanding of the foraging behaviour of ruffe, with a view to understanding its potential impact as an introduced species in Loch Lomond. The specific aims were:

- To examine anti-predator adaptations in a key prey species (*A. aquaticus*), looking in particular at the trade-off between seeking shelter and foraging in a potentially dangerous environment and how this is influenced by reproductive status (Chapter 2).

- To examine the possibility that *A. aquaticus* can detect chemical cues from potential predators and adjust its shelter seeking accordingly (Chapter 3).

- To make direct behavioural observations on the behaviour of ruffe when foraging in the dark, and to test the possibility that they can use non-visual cues to detect live concealed prey (Chapter 4).

- To make direct behavioural observations on interactions between ruffe and *A. aquaticus* when visual cues were available, examining the effects of shelter on these interactions, identifying anti-predator tactics in *A. aquaticus* and investigating prey selection by size and reproductive status (Chapter 5).

- To conduct a survey on ruffe collected from Loch Lomond, examining aspects of population structure, reproductive biology and the incidence of infection by macroparasites (Chapter 6).

Chapter 2

Foraging or hiding in *Asellus aquaticus*: influence of gender and reproductive status

Section I Preliminary study of the use of artificial shelter by *Asellus aquaticus*

2.1 Introduction

Asellus aquaticus is a benthic macroinvertebrate that is widely distributed across a variety of freshwater habitats. *A. aquaticus* feed on plant detritus (such as decaying leaves), and shelter amongst macrophytes, (Cummings and Klug 1979, Graça *et al.*, 1994a; Graça *et al.*, 1994b; Panov, 1988; Rask and Hiisivuori, 1985). *A. aquaticus* is a major prey species in the diet of ruffe (*Gymnocephalus cernuus*) throughout its range (Jamet and Lair, 1991, Kålås, 1995, Ogle *et al.*, 1995, Okland, 1980), including Loch Lomond (Adams and Maitland, 1998). *A. aquaticus* is also important as the intermediate host to the parasite *Acanthocephalus lucii*, whose definitive hosts are perch (*Perca fluviatilis*) and ruffe (Kennedy, 1974). It is known that *Acanthocephalus lucii* infection causes melanisation of *A. aquaticus* (Oetinger and Nickol, 1981) and that foraging perch eat significantly more infected individuals than uninfected, when presented with a choice of either (Brattey, 1983). The behaviour of *A. aquaticus* when infected with *A. lucii* has been studied to some extent (Lyndon, 1996), who found that infection with *A. lucii* did not influence behavioural responses to light and disturbance.

Refuges are very important to animals such as *A. aquaticus* in the wild, due to the high levels of predation pressure they experience (Rask and Hiisivuori, 1985). A field

based study of a freshwater Isopod *Lirceus fontinalis*, which lives in similar habitats to *A. aquaticus*, found dense groups of individuals on macrophytes more often than in exposed areas of sand and silt, (Holomuzki and Short, 1990). Indicating that shelter use in isopods is important, especially in avoiding predation, I designed this experiment to investigate whether *A. aquaticus* showed a preference for shelter, when offered a choice between artificial shelters and exposed tank areas.

Aims

The initial idea behind the experiment was to identify differences in shelter use between uninfected and *A. lucii* infected individuals. However no infected subjects were caught, therefore I was unable to do this. Instead shelter use by uninfected *A. aquaticus* was studied with a view to obtaining basic information on microhabitat choice in *A. aquaticus*, when in small groups. This would then allow me to design a more sophisticated set of experiments to investigate the effect other factors have on the behaviour of *A. aquaticus*.

- To develop a time budget for use of shelter by *A. aquaticus*
- To establish whether *A. aquaticus* aggregate on shelters
- To study whether individuals display preferences for particular categories of shelter, and if so what influences this.

2.2 Methods

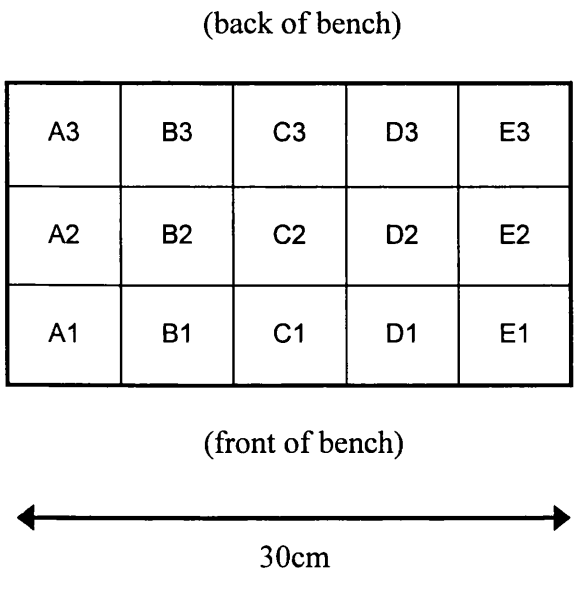
The experiments were carried out from June 1996 to September 1996 in an aquarium in the University of Glasgow. *A. aquaticus* were caught using dip nets, from a variety of locations within the Forth and Clyde Canal, Glasgow, over the duration of the experiments (Bishopbriggs 605,716; Glasgow Bridge 623, 731; and Cleveden Hill 557, 690. Map references from O.S. Land ranger 64). These were maintained in separate stock tanks according to their collection site, although no differences between the different populations were seen. All stock tanks contained a mixture of genders and reproductive states. The aquarium was maintained at 17°C with a light regime of 12 hours light/12 hours dark, all stock tanks and experimental tanks were kept in the same aquarium.

All experiments were conducted within plastic tanks, 300mm X 180mm, that were marked out with a grid, each square measuring 60mm X 60mm. Within each tank two artificial shelters were placed (within grid squares), in as random a combination as possible (See figure 2.1 for diagram of tank). The artificial shelters were constructed from black perspex and measured 60mmX60mm, they were supported by four legs (measuring 10mmX10mmX10mm), and were fixed into position with perspex cement. There were four experimental tanks, each in a line next to each other along a well, but unevenly lit, aquarium bench.

Both shelters used were identical in every way, with the exception of their position within the tank. Each tank contained 15 grid squares, which were either adjacent to 1side, 2 sides or no sides of the tank (figure 2.1). Shelter positions were changed with each run. Ten *A. aquaticus* (which were selected at random from our stock tanks) were introduced to each tank at a point roughly equidistant from the two shelters, to avoid shelter bias. Observations were made every 10 minutes for the first three hours and every thirty minutes up to a limit of 6 hours. With each recording, notes were made on the number of individuals in each grid-square and whether they were in or out of shelter. The experiment was repeated a total of 27 times using a variety of shelter positions.

Figure 2.1

Diagram of experimental tank (with grid references)



Data analysis

The data in this set of experiments was not normally distributed and therefore was subject to non-parametric analysis. Temporal differences were found with respect to shelter use after analysis with Kruskal-Wallis test, and therefore the initial 90 minutes of data collected has been excluded from all subsequent analysis. Aggregation was analysed using a Wilcoxon matched pairs test. Shelter preferences were examined with a series of Kruskal-Wallis tests. Data analysis was carried out in Excel version 6, and with statistical packages SPSS version 8 and Minitab version 11.

2.3 Results

Use of shelters over time

Figure 2.2 shows the median number of individuals in shelter at 30 minute intervals. Temporal differences with respect to shelter use were analysed using a Kruskal-Wallis test, and showed that the number of individuals using shelter increased significantly over time, ($H= 56.12$, $D.F= 13$, $P<0.001$). This temporal effect levelled off by 90 minutes. The first 90 minutes (inclusive) of data were therefore excluded from subsequent analysis, to stop any temporal effects influencing the data. All the data used in subsequent analysis were the median percentages of individuals in each shelter from 120 to 360 minutes.

Aggregation on shelters

To ascertain whether there were differences in the numbers of individuals found on each shelter within the tank, the data were sorted into 2 groups; with each shelter being assigned an arbitrary number in order to distinguish it from the other. A Wilcoxon Signed Ranks Test, showed that *A. aquaticus* groups consistently choose one shelter over the other, $Z= -3.626$, $N= 19$, $P= <0.001$, on which to settle (Figure 2.3). Therefore *A. aquaticus* appear to aggregate, choosing one shelter in preference to the other.

Nature of shelter preferences

Individuals appear to exercise choice when deciding which shelter to occupy, and it is possible that the position of a shelter within the tank influences this.

Figure 2.2

Temporal differences in shelter use by *A. aquaticus*

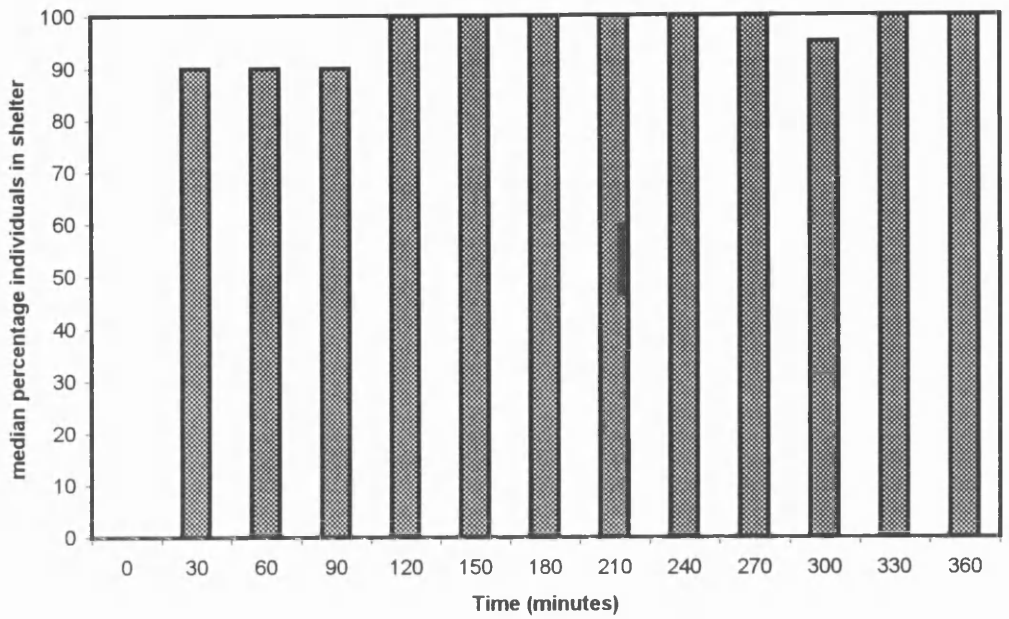
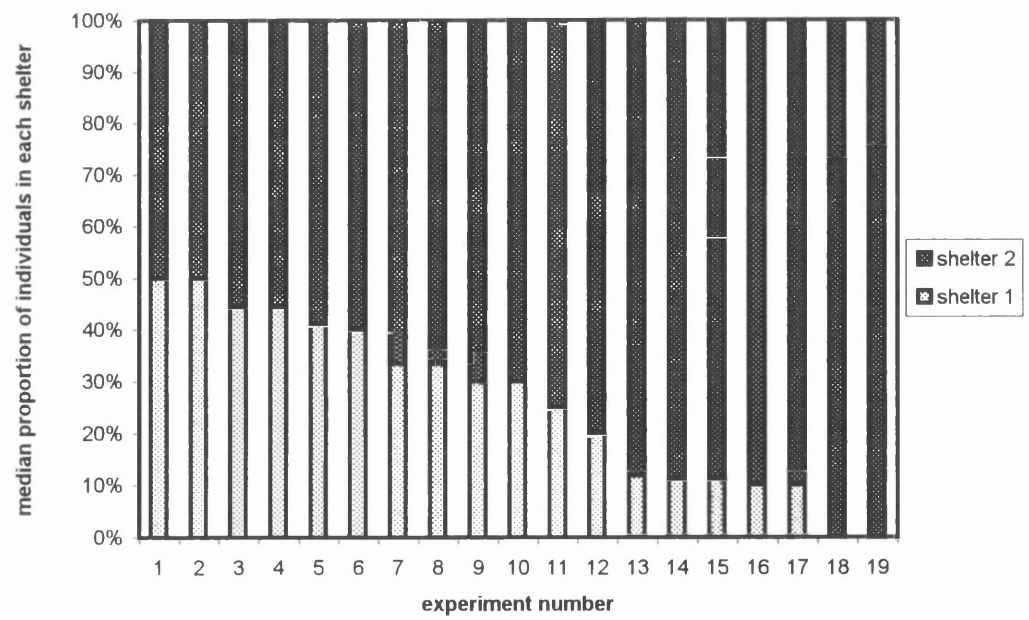


Figure 2.3

Distribution of individual between both shelters



A Kruskal-Wallis test was carried out in order to discover whether shelter position was important in shelter preference. The median proportion of individuals in shelter was used, along with codes to indicate the precise position of each shelter in a tank. It was found that the position of some shelters attracted significantly more individuals than others, (H=25.77, d.f=9, P=0.002), (Figure 2.4), clearly shows that certain shelter positions are preferred. However most of the shelter positions had very few replicates, (maximum of 9, minimum of 3), making further analysis into preferences for specific shelter positions difficult.

To find what factors were determining preference of shelter position, the median proportion of individuals in each shelter was compared with the number of sides of the tank adjacent to each shelter. A Kruskal-Wallis test found that there was no significant difference between the number of individuals present on shelters placed next to 1, 2 or no sides (H= 5.40, d.f= 2, P= 0.067). Although there appears to be a marginal preference for shelters adjacent to tank sides, Figure 2.5

To ensure that the non-uniform lighting across the four experimental tanks did not influence shelter use, a Kruskal-Wallis test was used to compare the median proportion of individuals in shelter for each experimental tank. There was no significant difference between the median proportion of individuals in shelter (H= 0.14, d.f= 3, P= 0.986), demonstrating that the position of experimental tank did not exert any effect on shelter use.

Figure 2.4

Shelter use by *A. aquaticus*, preferences for different shelter positions

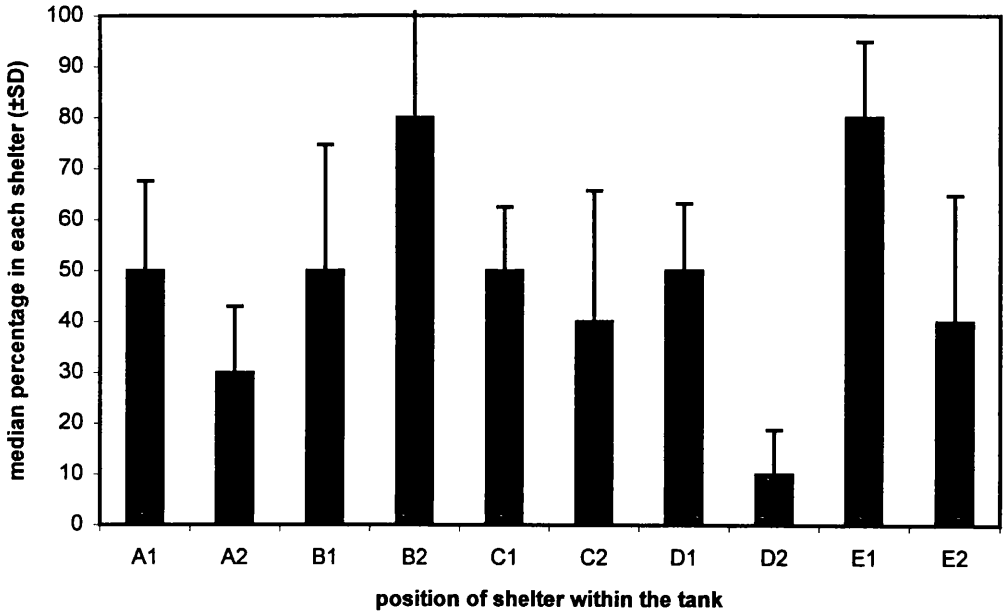
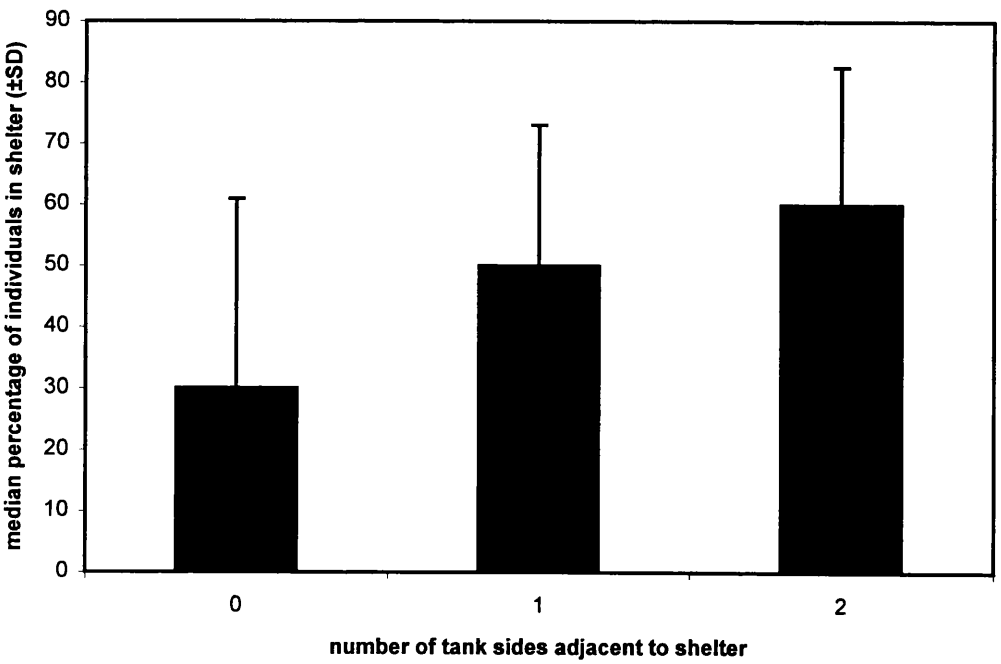


Figure 2.5

Influence of adjacent tank sides on shelter use in *A. aquaticus*



2.4 Discussion

Shelter use

The results clearly indicate that when given a choice between artificial shelters or a bare tank, *A. aquaticus* display preferences for shelter. They also aggregated within one shelter, even though two were provided. This directly relates to their behaviour in the wild, where they are found grouped together amongst vegetation (personal observation and Rask and Hiisivuori, 1985).

The temporal variation in shelter use is most likely due to a settling effect, when individuals are adapting to the experimental environment. Use of refuges in this experiment may have been exaggerated because of the relatively harsh tank environment. The tanks were fairly brightly lit and had no substrate, which made locomotion in the animals more restricted.

Shelter preferences

A. aquaticus displayed weak preferences for shelters adjacent to tank sides over the central position (with no tank sides), although this was not significant. It is possible that the exposed position of the central shelter made it less preferable, as it may have higher light intensities than the shelters next to the tank sides.

Conclusions

This study has shown that shelter use is not merely random, but leaves us unable to explain why *A. aquaticus* show group preferences for one shelter over another.

Possibly factors such as proximity to the shelter and direction an individual is facing when first introduced to the tank environment is influential in shelter choice. It is also possible that the presence of a small number of individuals in a shelter attracts others. This may be especially true if males are searching for mates.

The aim of this experiment was establish extent and time scale of shelter use as a guide for designing further experiments. On the basis of these data a further study into time partitioning and risk assessment in this species was conducted (Chapter 2, section II).

Section II Influence of gender and reproductive status on the trade-off between foraging and hiding in *Asellus aquaticus*

2.5 Introduction

Trading-off predation risk for other needs

In daily life every animal makes a variety of decisions regarding a wide range of basic requirements. Many animals live under a constant threat from predation, therefore it is essential that adequate assessment of potential risks can be balanced with other requirements. Individuals are forced to compromise their safety in order to forage (Lima and Dill, 1990; Pierce, 1988; Sih, 1992), attract mates (Endler, 1987; Tuttle and Ryan, 1981) and defend their territories and young, (Kruse, 1986). In situations like these, responses to perceived risk may decrease when balanced with necessity. Many studies have shown that animals have the ability to assess both predation risk and the costs of avoiding predation (Dill, 1987; Lima and Dill, 1990).

Behavioural adaptations that reduce predation risk

Species use a diverse range of strategies in order to reduce predation risk. Some individuals do this by foraging nocturnally, which can be influenced by factors such as moonlight. Deer mice, (*Peromyscus maniculatus*) reduce foraging activity on bright moonlit nights in order to reduce predation risk (Clarke, 1983). Others limit themselves to foraging in areas with cover, although this may prove less profitable (Price *et al,* 1984; Bowers, 1988). Daily migrations can also be used as a predator avoidance tactic, as in *Daphnia galeata mendotae* and *Mysis relicta*, which migrate to

the upper water column at night (Janssen, 1978; Janssen and Brandt, 1980; Schulze and Brooks, 1987; Young and Watt, 1993). This is not an entirely successful method of predator avoidance, as the alewife (*Alosa pseudoharengus*), a zooplanktivorous fish also migrates at night, utilising non-visual cues in order to forage (Janssen *et al.*, 1995).

The ability to detect the presence of predators allows individuals to assess the potential risks of emerging from refuges, and therefore to adjust behaviour accordingly (Lima and Dill, 1990; Fischer *et al.*, 1987, Holomuzki, 1986, Heads, 1985). Damselfly larvae switch to less profitable feeding areas when predators are present in profitable ones (Heads, 1986; Wellborn and Robinson, 1987) and can adapt behaviour according to the predator from which they are at risk. A study by McPeek, (1990) examined the behaviour of damselfly larvae (*Enallagma*) from different populations. Individuals from lakes without fish performed conspicuous displays, in contrast to those from lakes containing fish predators; these individuals were less active and rarely displayed. Individuals from both populations reduced activity in the presence of predators, however those from lakes containing fish evaded predation by *Aeshna* (dragonfly larvae) less effectively than individuals from lakes where fish are absent. This suggests that anti-predator behaviours are only useful if directed at certain predators. Freshwater snails employ different predator avoidance behaviours when under threat from crayfish or fish predators (Turner *et al.*, 1999).

Predation risks associated with reproduction

For all species reproduction incurs a variety of costs. One of these is the increased vulnerability to predation (reviewed in Magnhagen, 1991), that attracting mates (guppies Endler, 1987) and mate guarding (waterstriders Rowe, 1994) can bring. Simply the task of seeking out a mate can greatly increase the risk of predation (moths Acharya and McNeil, 1998; cicadas Gwynne, 1987). Vulnerability to predation often increases in females when gravid, brooding or pregnant (Brodie, 1989). Female *Daphnia* become heavily pigmented when carrying eggs, (ephippia), making them more conspicuous to visual predators, which can feed selectively on gravid females (Mellors, 1975). Gravid female lizards (*Eumeces laticeps*) suffer reduced stamina and running speed under laboratory conditions, and reduce their activity in the wild, as a method of decreasing predation risk (Cooper *et al.*, 1990).

Female isopods carry their developing young in ventral brood pouches. It has been shown that brooding females suffer from reduced agility as a result of this life history strategy (Lyndon, 1996), a possible consequence of this may be an increase in predation risk.

Predation on *Asellus aquaticus*

Asellus aquaticus is an important prey species for a variety of different predators, especially zooplanktivorous fish (Jamet and Lair, 1991; Kålås, 1995; Ogle *et al.*, 1995; Rask and Hiisivuori, 1985). They do not have any specially adapted means of escape from predators, other than either running away or remaining still, and therefore

it is essential that they avoid predation whenever possible. *A. aquaticus* and other benthic isopods reduce predation risk by foraging within cover, feeding on decaying vegetation, and spending much of their time hidden amongst macrophytes and plant detritus (Cummings and Klug, 1979; Graça *et al.*, 1994a; Graça *et al.*, 1994b; Panov, 1988; Panov *et al.*, 1995; Rask and Hiisivuori, 1985; Rossi and Fano, 1979; Rossi *et al.*, 1983). Foraging in sheltered areas in order to avoid predators is often associated with costs, as it may cause prey to forage in suboptimal areas. This behaviour is seen in the isopod *Lirceus fontinalis* (Holomuzki and Short, 1988; Holomuzki and Short, 1990). In the presence of predatory fish, individuals remained in macrophyte shelters, which are a poor source of food. Remaining in these refuges for extended time periods resulted in individuals having a lower growth rate. This is particularly costly to isopods, as fecundity in females is dependent on body size. Males also require a large body size to compete for mates and successfully mate-guard females.

Reproduction in *Asellus aquaticus*

Reproduction in *Asellus aquaticus*, as in many crustaceans, involves a period of pre-copulatory mate-guarding, as fertilisation only occurs directly after female moulting. Males select females on the basis of a combination of size and proximity to ecdysis (Ridley and Thompson 1979; Shuster, 1981) and may carry a single female beneath them for up to eleven days (often whilst fending off other males) until fertilisation can take place (Manning 1975, Unwin 1920). Once fertilised, females carry their developing young in a specialised brood pouch on the ventral surface of the thorax.

One may assume that this period of precopulatory mate-guarding could prove risky, leaving individuals more prone to predation because of a reduced mobility (Adams *et al.*, 1985). This is therefore one of the factors that may influence *A. aquaticus* in their choice of habitat during this period. It is known that females carrying broods have more difficulty righting themselves when up turned (Lyndon, 1996), which could cause difficulties when escaping predators. Individuals that are either mate-guarding or carrying broods should therefore try to reduce this risk, possibly by decreasing their activity or by seeking shelter.

Aims

Preliminary work on the use of shelter by *A. aquaticus* has highlighted the importance of shelter for this species (Chapter 2, Section I), but little is known about the influence of mating and reproductive status on behaviour in this species. The objective of the study was to describe habitat use in *A. aquaticus* individuals of different gender and reproductive status, when faced with a conflict between foraging and shelter seeking, and to examine the possible trade-offs between these. The specific questions to be addressed were:

- What is the time scale for adjustment to novel habitats?
- Are there consistent individual differences in habitat preference?
- Do *Asellus aquaticus* trade-off feeding against shelter?
- Does behaviour change with time or experience?

- Do different categories of *A. aquaticus* respond differently with regard to habitat?
- How does trade-off occur with respect to stage in the brooding cycle?

2.6 Materials and Methods

Collection and Husbandry

Asellus aquaticus were collected throughout June and July 1997, from the Forth and Clyde Canal, at a site near Bishopbriggs, Glasgow (OS ref: 605,716 Landranger 64). A pond net was used to catch *A. aquaticus* from amongst macrophytes at the canal bank. Individuals were picked out of the net and put into watertight storage pots, for transport back to the aquarium in Glasgow. At this point pairs in precopula and females carrying broods were separated from the other individuals.

A. aquaticus were kept in a series of stock tanks (300mm X 180mm), in an aquarium maintained at 17°C, with a light regime of 12 hours light, 12 hours dark. Decaying leaves were added to the stock tanks for food, and the tanks were aerated at a slow rate. Four different reproductive types and genders were used in the experiment. *A. aquaticus* were sorted into different groups which were kept in separate stock tanks. These groups consisted of:

- pre-copulatory mate-guarding pairs
- females carrying broods of unknown fertilisation date
- females carrying broods that share the same fertilisation date
- post-mated males
- females that had released their broods
- females sharing the same brood release date
- individuals of undetermined sex and juveniles

Throughout the breeding season (Spring and Summer), females carrying broods and pairs in precopula are highly abundant, and in the aquarium the stock tanks suffered from problems of mortality amongst post-mated males and post-brooding females, which is consistent with other semelparous species (Tuomi *et al.*, 1988).

The tank containing pairs in precopula was checked twice a day (morning and evening), for any pairs that had mated. Of these individuals, the females were kept in small tanks corresponding to the date of fertilisation and males were maintained in a separate tank. Brooding females of unknown fertilisation date were kept together until the release of their broods, they were then stored with other females of the same release date. Four reproductive types were used in the experiment: males, post-brooding females, brooding females and mating pairs; and therefore a technique for sexing *A. aquaticus* was developed.

Method used for ascertaining gender of *A. aquaticus*

Brooding females were identified by the presence of a ventral brood pouch containing eggs or developing young. Mate-guarding pairs were easily identified, as the male carries a female beneath himself. Post-brooding females were identified as individuals that had been carrying broods, but now had empty brood pouches.

Distinguishing between non-breeding males and females is more difficult. I therefore taught myself to do this, by a combination of examining individuals of known gender and with the aid of diagrams from Richardson, (1905). I made

sketches of the first pleopods and first legs of males and females (Figures 2.6 and 2.7), and used these as a guide for identifying the gender of individuals.

Marking

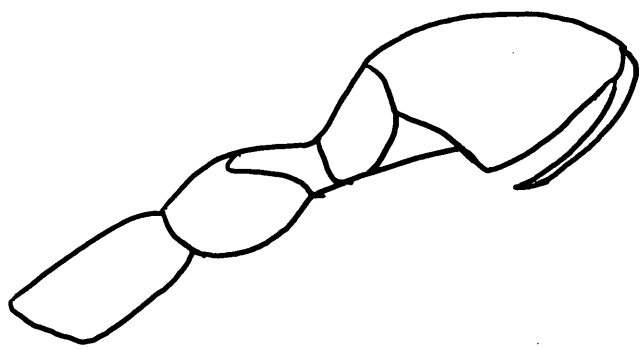
In order to examine the behaviour of individuals *A. aquaticus*, individuals from each reproductive category were randomly chosen and marked, in order to gather data on individual habitat preferences. Several considerations had to be made when devising a marking technique:

1. The marking medium must not dissolve in water.
2. It must persist for a reasonable length of time (up to two weeks).
3. It must not adversely effect the survivorship and behaviour of individuals.

It was necessary to apply the marks when *A. aquaticus* were out of water, and therefore I tested whether *A. aquaticus* could remain outside of water without their survival being reduced. When testing this we observed not only that *A. aquaticus* survive well outside of water, for at least up to 10 minutes (we did not test them for longer than this), but that many individuals remained fairly calm, and quickly resumed normal behaviour once returned to water. Once I had established that *A. aquaticus* could remain out of water for long enough to apply and dry the marks, we went on to try different marking materials.

Figure 2.6a

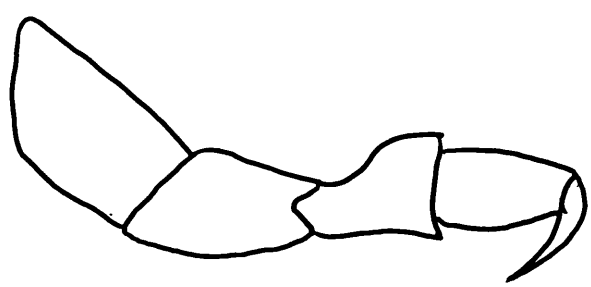
First leg of male *A. aquaticus.*



(Actual size: 1.5mm long)

Figure 2.6b

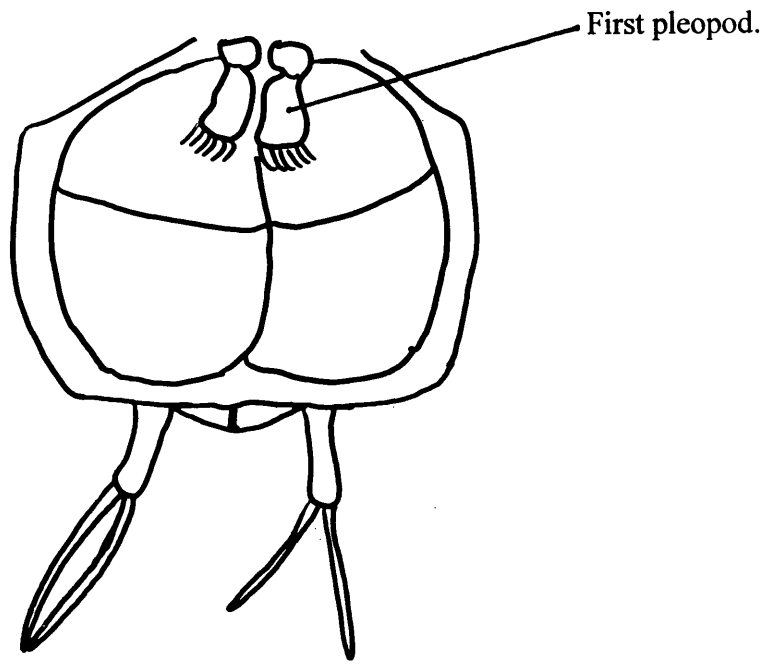
First leg of female *A. aquaticus.*



(Actual size: 1mm long)

Figure 2.7a

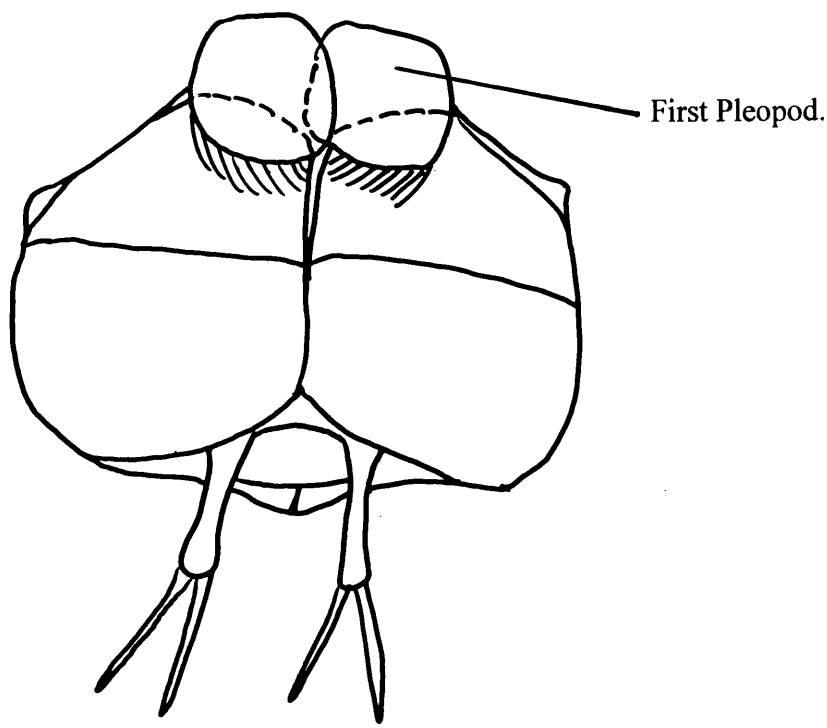
Abdomen of male *A. aquaticus.*



(Actual diameter: 2mm)

Figure 2.7b

Abdomen of female *A. aquaticus.*



(Actual diameter: 1.5mm)

A few different methods were employed before I found a truly effective one.

I knew that it would not be possible to use paints due to problems with dissolving, drying times and toxicity of gloss paints and therefore initially used solvent based correction fluid (white). This worked relatively well, as it dried quickly although it did not persist for very long (a couple of days at the most, mainly a few hours). Coloured correction fluid was then used, however these were not solvent based, and simply dissolved once individuals were returned to water. Normal white correction fluid was then dotted with coloured permanent markers to give a variety of coloured dots, with which to identify different individuals. This technique reduced the persistence of marks even further and therefore it was important to find a different marking material.

Solvent-based nail varnishes were then used, which proved ideal due to the quick drying properties of many newer brands. I was also able to get nail varnish in a wide variety of bright colours. Marks persisted fairly well on most individuals, although there was a continuous problem of some marks remaining for shorter times. The formula of nail varnish with the greatest persistence was Boots 17 advanced wear one coat nail polish, which luckily for me also came in a wide variety of garish colours. Marked individuals were observed to see whether survival was reduced or if behaviour altered, neither of these potential effects were observed.

Some marks did not persist for very long, however others remained for long enough to carry out several experimental repetitions on the same individuals.

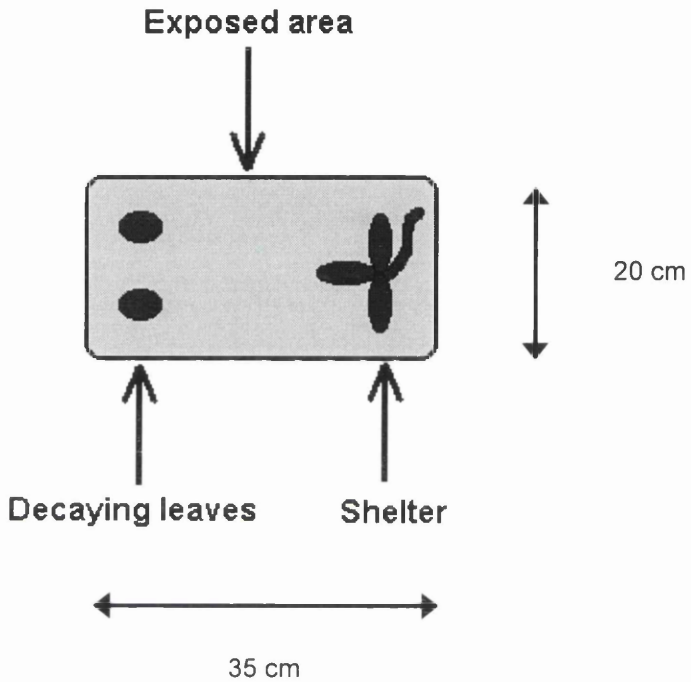
Experimental Method

This study was conducted between 10 June and 23 July 1997, in an aquarium at the University of Glasgow. The temperature range throughout the experimental period was between 16.8 and 17.8°C, with an average daily temperature of 17.3°C. The aquarium light regime was 12 hours of light and 12 hours of dark, with all the experiments being conducted during daylight.

This series of experiments were conducted in four glass tanks measuring 350 x 200mm. The bottom of each tank was filled to a depth of 30mm with washed building sand. The tanks were divided into three different areas, with a feeding area at one end, shelter at the other and an intermediate exposed area (diagram of tank, figure 2.8). The shelter consisted of a single artificial plant constructed of three textile leaves and a plastic coated wire stem. In the other end of the tank three leaves cut into circles of approximate diameter 30mm were secured into place by small toothpicks, to prevent individuals from crawling underneath. In order to allow individuals to feed in a less exposed area, this end of the tank was shaded by a black bag on two of its sides, and overhead by a grey plastic shade with small holes in to allow light through. These two distinct areas were separated by approximately 200mm of well lit sand, with no refuges. The leaves used in the experiment were collected from a large oligotrophic loch (Loch Lomond), and were in a state of decay. The different tank areas will be referred to as habitats from herein. Individuals were able to move freely between these habitats throughout the experiments.

Figure 2.8

Diagram of experimental set-up



There were four habitat classes, which depended on the position and behaviour of individuals. Habitat types: shelter, exposed, feeding and shaded not feeding. This last category refers to individuals which were present in the shaded feeding area, that were not feeding. An individual was only described as feeding if it was observed 'scraping' the surface of one of the leaf discs (or in some cases a toothpick), with its mouthparts.

At the beginning of each experiment, ten individuals were placed into the exposed area of each tank, half way between the shelter and feeding area. Data regarding the tank position of individually marked *A. aquaticus* were collected, in addition to the overall numbers of individuals in each habitat. Data were collected every 10 minutes for the first 3 hours, and every 30 minutes thereafter. By using this method, the intention was to collect information regarding the habitat preferences of both individuals and groups of *A. aquaticus,* of different reproductive status over time.

Experimental repetitions on the same individuals were carried out when possible (when marks persisted for long enough). Data was also collected on females of known fertilisation date carrying broods, relating to the number of days they had been carrying their broods, and when possible after they had released them. Repetitions of this experiment were carried out until there were 68 data sets available on separate tanks, full details of repetitions are given in Tables 1 and 2.

Efforts were made throughout to complete the same number of repetitions for each group of *A. aquaticus*. This was made difficult, due to the different relative abundance of each group in the wild and the high mortality of post mated individuals. Also experiments of pairs in precopula were difficult to carry out in high numbers, because handling of pairs often ended mate-guarding (breaking up the pairs), these individuals were particularly difficult to mark. The data from some tanks have also been discarded as they contained a mixture of groups, which may have affected individual behaviour.

Data analysis

The number of repetitions of the experiment and the numbers of marked individuals studied are shown in table 2.1. The data were analysed using Microsoft Excel version 5, Minitab version 11 and SPSS version 8. The data were not normally distributed and therefore all the statistical tests used are non-parametric. Multiple comparison tests were carried out according to the formulae and data tables in Siegel and Castellan (1988).

Table 2.1a

Number of repetitions conducted for each reproductive category

Reproductive category	number of repetitions
males	12
brooding females	24
post-brooding females	8
mating pairs	9
miscellaneous	10
mixed	5
Total	68
Total used in analysis	53

Table 2.1b

Number of marked individuals tracked for more than 1 experiment

repetitions	males	brooding females	post-brooding females	mating pairs
1	42			10
2	5	12	7	7
3	7	9	2	3
4	1	7	1	0
5	4	1	0	0
6	1	3	0	0
7	0	2	0	0
8	3	0	0	0

Table 2.1c

Number of individually marked *A. aquaticus* used

Reproductive category	number of individuals
males	180
brooding females	345
post-brooding females	75
mating pairs	120

2.7 Results

Temporal differences within and between trials

Initially it was important to establish whether habitat use changed over time, due to the effects this could have on subsequent analyses. Figure 2.9 shows the temporal differences in habitat use over the experimental period. A Spearmans Rank Correlation on shelter use by marked individuals (regardless of gender), showed a strong positive correlation (r_s =0.493, N= 714, P<0.01), with individuals increasing their use of shelter as the experiment progressed. This test was repeated, using the data for the proportion of time spent by individuals, for each reproductive category: males (r_s =0.456, N= 177, P= 0.01), brooding females (r_s =0.606, N= 343, P= 0.01), post-brooding females (r_s =0.571, N= 74, P= 0.01) and mating pairs (r_s =0.521, N= 120, P= 0.01); demonstrating that temporal differences occur with respect to use of shelter in all categories (see figure 2.10). This is possibly due to a settling effect at the beginning of the experimental period. There is a levelling out of changes in habitat use after 90 minutes (figure 2.9), and therefore all subsequent analyses use data from 120 minutes onwards.

Behavioural differences between the reproductive categories

Inter-category differences exist in the use of all habitats (Kruskal-Wallis test N= 396): feeding, H=12.13, P 0.007; shelter, H=51.55, P<0.001; exposed, H=45.30, P<0.001; and shaded not feeding, H=66.00, P <0.001; suggesting that reproductive status and gender influence habitat preferences (figure 2.11). To locate these differences two-tailed non-parametric multiple comparisons tests were used,

Figure 2.9

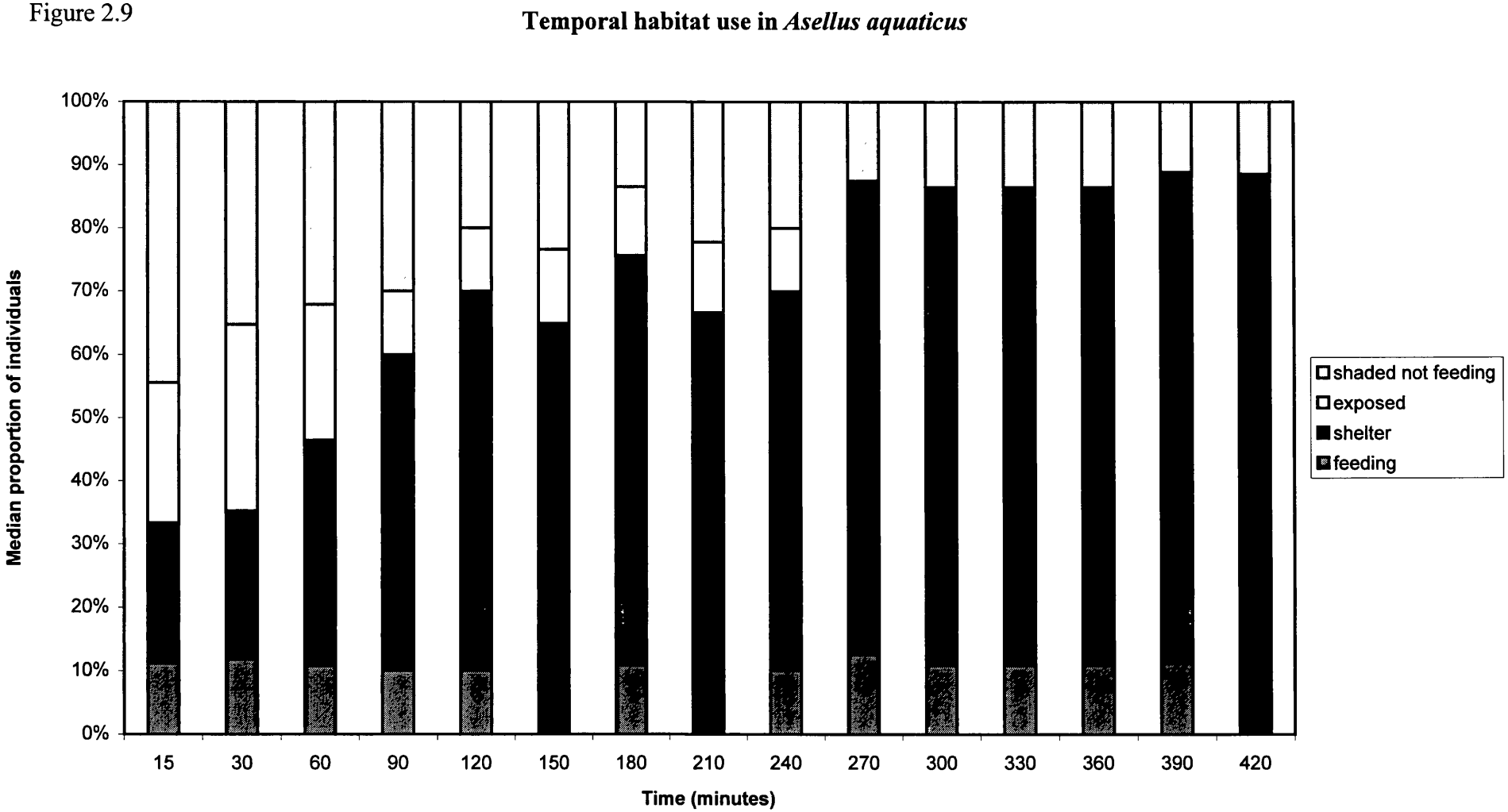

Temporal habitat use in *Asellus aquaticus*
Median proportion of individuals
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
15
30
60
90
120
150
180
210
240
270
300
330
360
390
420
Time (minutes)
shaded not feeding
exposed
shelter
feeding

Figure 2.10 Temporal differences in the median percentage of time spent by individuals of different reproductive categories in shelter

Figure 2.10a Males

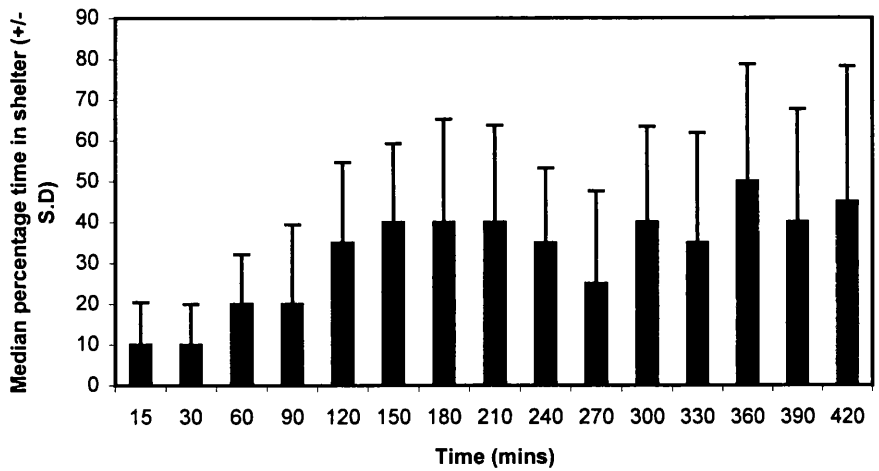


Figure 2.10b Brooding females

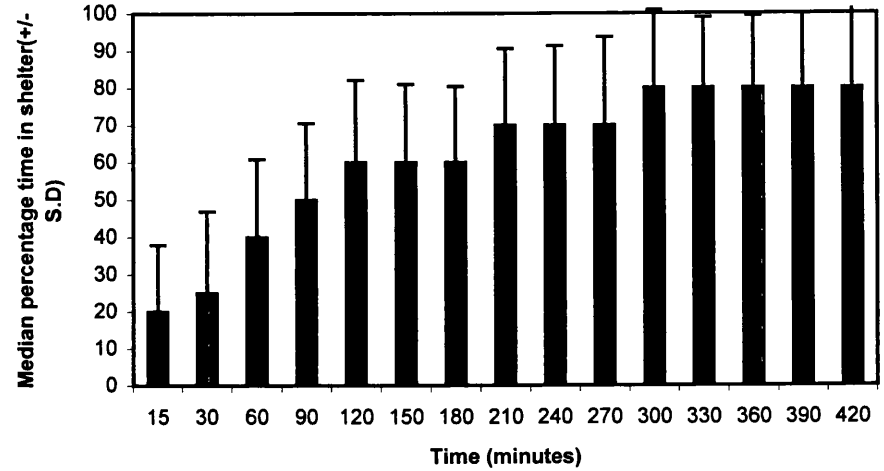


Figure 2.10c Post-brooding females

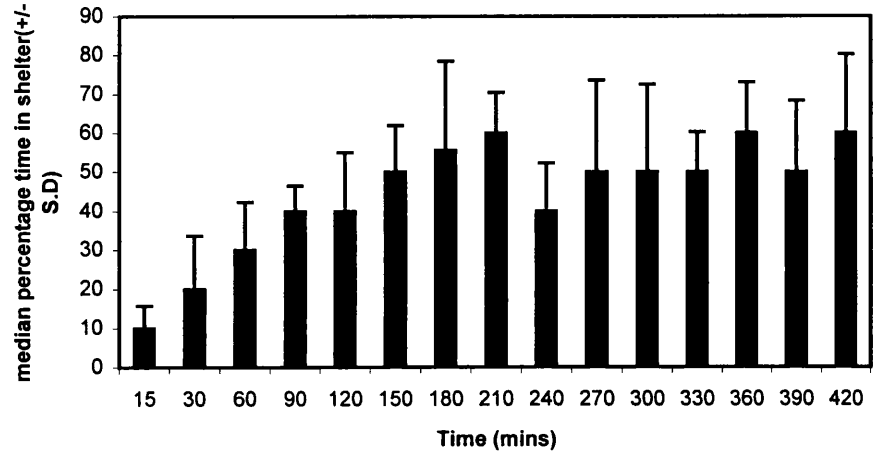


Figure 2.10d Mate-guarding pairs

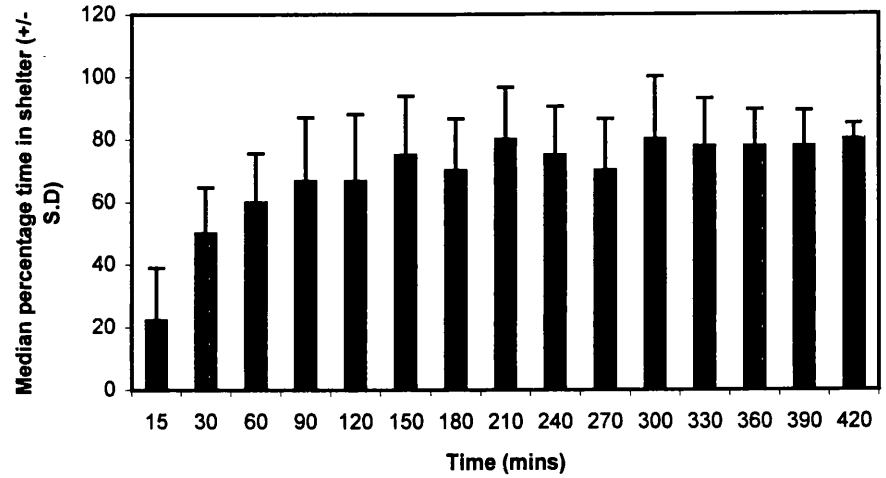
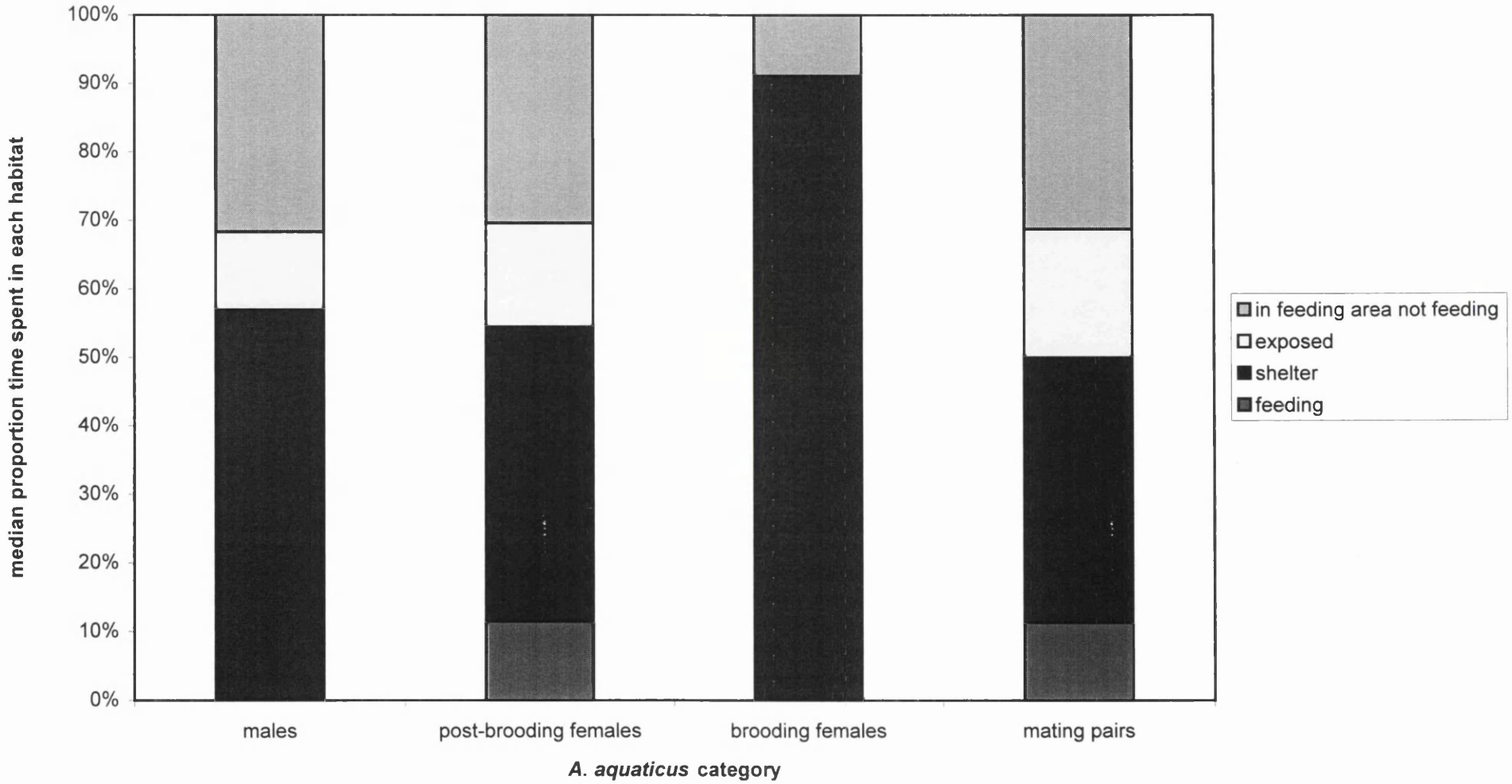


Figure 2.11

Habitat use by different *A. aquaticus* reproductive categories.



the results of which are in table 2.2. These tests highlighted numerous differences in shelter preference between the different reproductive categories. Brooding females and mate-guarding pairs spent significantly more time in shelter than males and post-brooding females, which were more active, being present in exposed areas more often. Although the use of the exposed area did differ significantly between categories, generally all groups avoided it. Although relatively little time was spent feeding, post-brooding females spent significantly more time feeding than males or brooding females.

Temporal differences between trials

In order to examine whether individual *A. aquaticus* behaved consistently with respect to habitat use when introduced to the experimental set-up on several occasions, the proportion of time each individual spent in each area, was examined, within reproductive categories. Individuals from all reproductive categories were consistent in their use of different habitats between experimental repetitions, with the exception of brooding females, (d.f= 33, feeding H=65.56, P=0.001, shelter H=58.61, P=0.004; exposed H=52.95, P=0.015). Because this trend for differences in individual behaviour are confined to brooding females alone, it is possible that behaviour in this group is influenced by stage in the brooding cycle; or perhaps this is simply some type of sequence effect.

To establish whether prior experience of the experimental set-up altered behaviour over the complete experimental period, data from marked males were analysed (Kruskāl-Wallis), significant differences existed in feeding (H=17.62, d.f= 9,

Table 2.2

Results from non-parametric two-tailed multiple comparisons tests (based on data from Kruskal-Wallis tests), comparing the use of different microhabitats by each reproductive category. P= 0.05.

A. aquaticus group	Use (a habitat) more than	*A. aquaticus* group
Post-brooding females	feed	Males
Post-brooding females	feed	Brooding females
Brooding females	use shelter	Males
Brooding females	use shelter	Post-brooding females
Mating pairs	use shelter	Males
Mating pairs	use shelter	Post-brooding females
Males	are exposed	Brooding females
Males	are exposed	Mating pairs
Post-brooding females	are exposed	Mating pairs
Brooding females	are exposed	Mating pairs
Males	in feeding area not feeding	Post-brooding females
Males	in feeding area not feeding	Brooding females
Males	in feeding area not feeding	Mating pairs
Post-brooding females	in feeding area not feeding	Brooding females

P=0.04) and shelter use (H=18.0, d.f=9, P=0.035). No differences were seen with respect to time spent in the exposed area (H=11.42, d.f=9, P=0.248) or in the shaded area (H=13.64, d.f=9, P=0.136). Further analysis failed to locate any significant differences, (two tailed multiple comparisons tests for significance level P=0.05); possibly because of small sample sizes or the large variation in habitat use by different individuals. Small sample sizes prevented data from females and mating pairs being analysed in the same way. Although not significant, weak trends could be seen in the data, with a tendency for individuals to spend more time in shelter and less time feeding in the first 3 days, with a rise in feeding on day 7. The fluctuations in preferences for shelter or feeding may be due to experience of the experimental set-up, and could also be influenced by hunger.

Individuals differences in behaviour within reproductive categories

Intra-group differences with respect to habitat use were found in brooding females (Kruskal-Wallis, N= 34: feeding, H=66.30, P=0.001; shelter, H=60.30, P=0.004; exposed H=10.29, P=0.013 and shaded not feeding, H=5.96 P=0.015). No other reproductive category showed significant differences in the behaviour of different individuals, despite the relatively variable data. It is therefore possible that stage in the brooding cycle effects habitat choice.

Stage in the brooding cycle

The data were re-analysed comparing individual females at five different points in the brooding cycle (figure 2.12). Differences in habitat use with brooding stage were confirmed (Kruskal-Wallis N= 108: feeding, H=27.66, P<0.001, shelter,

H=30.35, P<0.001; exposed, H=10.75, P=0.03; and shaded not feeding, H=15.36, P=0.004, multiple comparison tests located these differences, which are shown in detail in table 2.3. Females in the middle stages of brooding, spent significantly more time in shelter than those in the last few days of brooding, or just after brood release. Females in the last stage of brooding spent more time in the feeding area (without feeding), than those in the middle of the cycle. Just following brood release, the proportion of time spent feeding greatly increased, and was significantly higher than females mid-cycle.

Figure 2.12

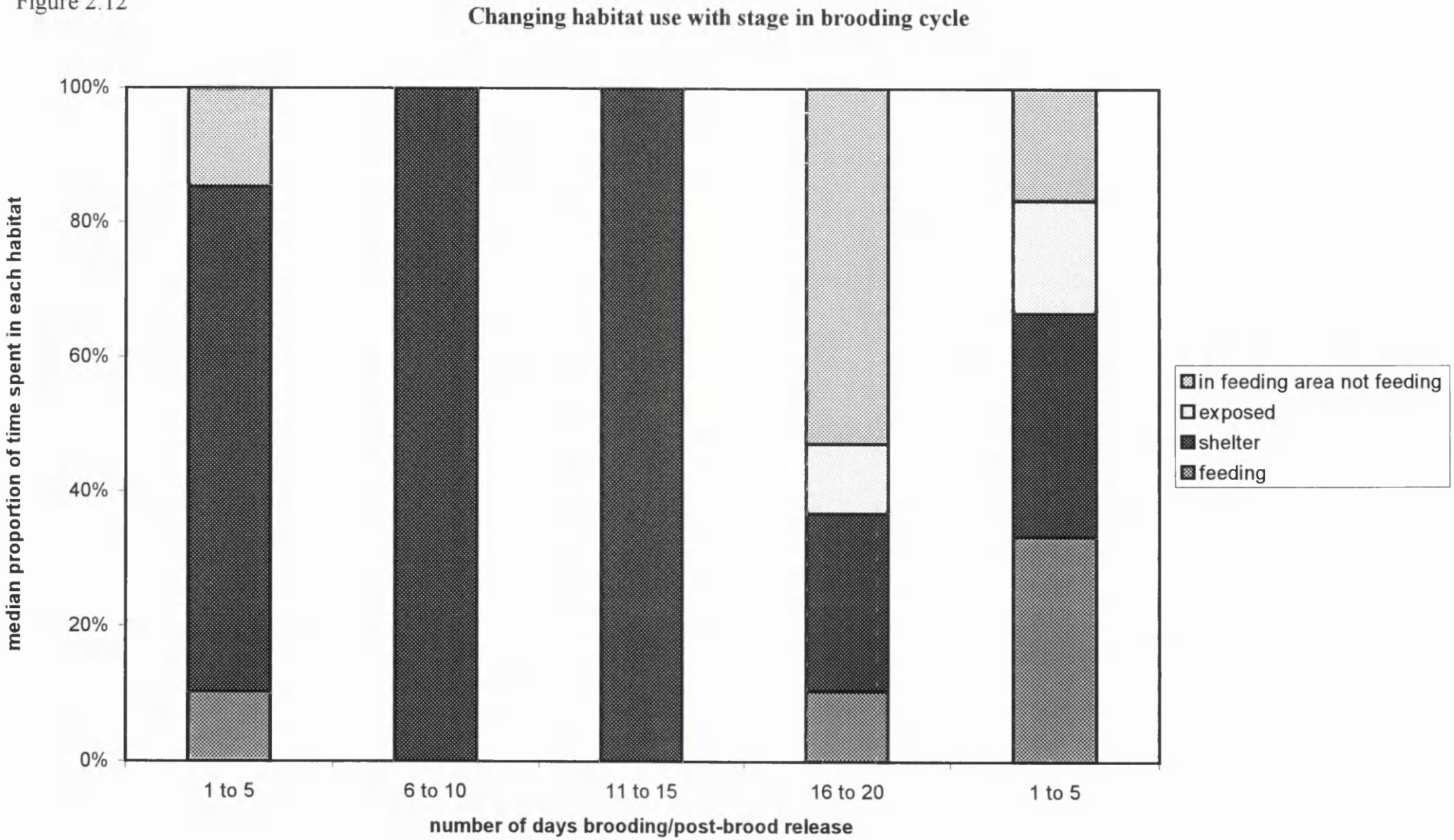

Changing habitat use with stage in brooding cycle
median proportion of time spent in each habitat
100%
80%
60%
40%
20%
0%
1 to 5
6 to 10
11 to 15
16 to 20
1 to 5
number of days brooding/post-brood release
in feeding area not feeding
exposed
shelter
feeding

Table 2.3

Results from two-tailed non-parametric multiple comparisons tests (based on data from Krusal-Wallis tests) comparing microhabitat use by females in different stages of brooding. P= 0.05.

Days into brooding	Use (a habitat) more than	Days into brooding
Brood released (less than 5 days)	feed	6 to 10 days
6 to 10 days	use shelter	16 to 20 days
6 to 10 days	use shelter	Brood released (less than 5 days)
11 to 15 days	use shelter	Brood released (less than 5 days)
16 to 20 days	in feeding area not feeding	6 to 10 days
16 to 20 days	in feeding area not feeding	11 to 15 days

2.8 Discussion

I investigated whether individuals in different reproductive states perceived risk in the absence of any direct predatory stimulus, and by doing so hoped to gain insight into behavioural modifications induced by reproduction in this species. Most individuals did not behave differently between experimental repetitions with the exception of brooding females. Although prior experience to the tank environment appears to influence behaviour, the large variance in habitat use by individuals prevented us examining this further.

Habitat preferences in different reproductive categories

A wide variance in the use of habitats by individuals of the same group was seen. However significant intra-group differences only occurred in brooding females, suggesting that they are subject to fluctuating demands which influence their behaviour. Previous studies on other isopod species have shown that males and females display preferences for different microhabitats (Holomuzki and Short, 1990; Jormalainen and Shuster, 1997; Merilaita and Jormalainen, 1997), but did not investigate behavioural changes exhibited during the brooding cycle in females.

Influence of brooding stage on behaviour

A brooding stage effect was confirmed when habitat use was compared with point in the brooding cycle. Females in the earliest brooding stages showed a trend of feeding more than individuals in the middle stages, and it is possible that these individuals were using the time when brood mass is still relatively low to increase energy stores

for the brooding process. The phenomenon of females increasing foraging rates before reproduction has been observed in the marine isopod *Idotea baltica*, as a method of storing energy reserves for reproduction, (Tuomi and Jormalainen, 1988; Tuomi *et al.*, 1988).

Females used shelter more, and fed less in the middle stages of brooding than in later stages and after brood release. This suggests that females perceive higher levels of risk in the mid-cycle without being nutritionally limited to the same extent as those nearing the end of brooding. These results show that food is a limiting factor for female isopods, (a point rarely made in previous studies), and that it becomes more important as brooding progresses, with females feeding more frequently after brood release. It is also possible that food abundant areas are important to females in the final brooding stages, to provide their young with profitable habitats once released. Observations of females that released their broods in experimental tanks showed juveniles to be present in higher numbers on the leaves rather than on the sand. Juveniles were never found in shelter.

Exposed areas were not used differently over the brooding period, indicating that a general avoidance of this area was universal, which supports the idea that females in the final stages of brooding may be seeking out suitable areas for brood release. Few studies have examined the behaviour associated with brood release in invertebrates, and so it is difficult to put these findings into context.

This study has shown that habitat preferences were influenced by reproductive status, suggesting that individuals adapt their behaviour according to the changing demands and priorities driven by reproduction. Thus it was shown brooding females and mating pairs use shelter more and are less active than both non-mating males and females, there are two main explanations for this result. Firstly both gravid females and mating pairs may be at a higher risk from predation, due to the reduced mobility that both these reproductive strategies incur (Adams *et al.*, 1985; Cooper *et al.*, 1990; Rowe, 1994). Secondly it is possible that both brooding and mate-guarding increase energetic demands, and by reducing activity during these periods reproductive costs may decrease (Sparkes *et al.*, 1996).

Energetics of reproduction

Studies on other freshwater Isopods (*Lirceus fontinalis*) have highlighted that precopulatory mate-guarding is energetically costly to males (Sparkes *et al.*, 1996). It could not be proven that this was due to carrying females, and instead highlighted the importance of food deprivation in males when in refuges during this phase. They also suggested, that males use shelter to avoid competing conspecifics which could take over the female. Competition between males for females also occurs in *A. aquaticus* (Ridley and Thompson, 1979), and was also observed in our mixed stock tanks, it is therefore likely to be important for male *A. aquaticus* when choosing a habitat.

A combination of these factors is important when making decisions regarding shelter use. Foraging will increase risk at a time when energetic demands are high, so to reduce both risk and energy expenditure, individuals remain in shelter. Activity alone

can increase the risk of predation from visual and motion sensing predators (Heads, 1985; Janssen *et al.*, 1995; McPeek, 1990).

Feeding

males

The marine isopod *Idotea baltica* also displays inter-gender differences in microhabitat preference (Merilaita and Jormalainen, 1997). Males and females preferentially use different areas of *Fucus vesiculosus* (bladder wrack) thalli, with males locating themselves on the younger upper parts and females found on the lower areas where vegetation was at a higher density. Indicating that females are trading-off the high risk, nutritious upper areas of thallus in preference for reduced predation risk. The study attributed this trade-off to the different reproductive strategies of each gender. Where females must increase their survival over the long period required for successful brood production, and males need to increase their body size by feeding at a higher rate, to give them an advantage when competing for mates and mate-guarding. A previous study has highlighted that size in male *A. aquaticus* is essential for securing highly fecund females (Ridley and Thompson, 1979), as larger females produce larger broods. A large body size in males is essential in order to effectively mate-guard females against the attempts of other competing males. Merilaita and Jormalainen, 1997 did not distinguish between brooding and post-brooding females, and while our results for brooding females support their findings, our data on feeding in males and post-brooding females is very different. Male daytime foraging could be reduced by the search for mates, which in this experiment appears to be continuous,

with males attempting to mate-guard other males. This may explain the high incidence of males in exposed areas.

females

Considering the many studies that highlight the importance of nutrition for males during mate-guarding, the nutritional status of brooding females has been largely neglected. Females spend a very large proportion of their time in shelter when brooding, and in our experiments they were unable to feed in these areas. Brooding (which was observed up to a maximum of 19 days) may be nutritionally costly due to the elevated feeding levels displayed by post-brooding females. Whether like the males in Sparkes *et al.* (1996), they were replenishing lost food stores from being unable to feed whilst in shelter, or whether a greater cost was incurred by the increased demands of carrying broods we cannot say.

Cannibalism

Another study has suggested different reasons for this variance in habitat selection by the sexes. Microhabitat segregation by males, females and juveniles in the freshwater isopod *Thermosphaeroma thermophilum* has been attributed to avoidance of cannibalism, which is common in this species (Jormalainen and Shuster, 1997). Males cannibalise both adult females and juveniles, with females cannibalising juveniles. Females and juveniles spend more time on vegetation avoiding males, who prefer the substrate.

A. aquaticus also cannibalise each other when in mixed stock tanks (personal observation), however this has not been studied in any detail, and we cannot confirm that males selectively cannibalise females. During preliminary studies we witnessed cannibalism on two occasions, within tanks containing a mixture of non-mating and non-brooding individuals of different genders. The data used in the analysis was taken from individuals in tanks containing only one group (mixed tanks were used during preliminary stages only). Therefore threat of cannibalism from other genders would not have applied in this case.

Conclusions

This study highlights that even in the absence of a predatory stimulus, individuals engaged in mate-guarding and females carrying broods appear to perceive a higher degree of risk than non-mating individuals. This investigation cannot confirm that high levels of shelter use by these individuals is entirely due to potential risk from predators, as energetic costs may also play a role. With individuals using refuges as areas where they can remain inactive for long periods to conserve energy. Shelters for mate-guarding males may also be important in seeking refuge from competing males that could attempt to take-over a female. We have also shown that brooding stage can greatly influence habitat preferences, and that reproduction for female *A. aquaticus* may carry high energetic costs due to reduced foraging activity. This was highlighted by the great increase in foraging, that females experience at the cessation of brooding. It is also possible that females attempt some form of post-release brood care by the observations we made of females nearing brood release leaving shelter in favour of the feeding area, although this could just be a product of female hunger.

Chapter 3

An examination of chemical cues from fish predators, on shelter seeking in *Asellus aquaticus*

3.1 Introduction

Background to study

This study was designed as a follow up to my previous investigation of microhabitat preferences in different reproductive categories of *A. aquaticus* (chapter 2). I already knew that the use of different habitats, changes with reproductive status of both male and female *A. aquaticus*, and I aimed to investigate whether environmental stimuli influenced this behaviour under laboratory conditions.

A previous study highlighted the reactions of *A. aquaticus* to a variety of environmental stimuli (Lyndon, 1996), finding that *A. aquaticus* avoid both disturbance and light. These responses would enhance predator avoidance, as fish cause water disturbance and many are visual predators requiring light in order to forage efficiently. I was interested in taking the study of responses of *A. aquaticus* to cues indicative of predators further, by investigating whether they could utilise chemical cues in the surrounding environment to assess potential risks.

Olfaction in the aquatic environment

Many studies have investigated the use of both visual and auditory cues in detection of predators, but currently there is a growing number examining the use of chemosensory cues in this role (review in Kats and Dill, 1998). Chemosensory cues are especially important to aquatic animals that live in areas of low light and high turbidity, such as the benthos, and where water currents may hide mechanosensory

cues of disturbance. Aquatic environments are well suited to the dissemination of chemical information as substances can be carried in solution. This is true of the mucus secreted by fish to protect their scales. This exudate has been shown to give a variety of information to other aquatic animals (Boriss *et al.*, 1999).

Predators using chemical cues to detect prey

A variety of aquatic predators are known to utilise chemical cues in the search for prey. This is important, as often in aquatic habitats, visibility can be reduced by low light intensity at depth, turbidity and the presence or macrophytes, in which prey can shelter. When exposed to water from the Isopod *Asellus aquaticus* placed upsteam, *Gammarus minus* (a known predator of *A. aquaticus*) moved towards the source of the chemical stimuli (Bengtsson, 1982). Predatory crayfish (*Orconectes rusticus*) locate their snail prey (*Physa gyrina*) by searching the substrate for chemical cues (*Dewitt et al.*, 1999). Many fish species utilise chemical cues from prey species, in the location of food sources (reviewed by Jones, 1992). For example, a study on the actively foraging cod (*Gadus morhua*) showed that they could detect chemical cues from mackerel baits from several hundred meters away (up to 698m) (Løkkeborg, 1998).

The use of chemical cues by aquatic prey species

This ability to utilise chemosensory cues, is not limited to predators. Many prey species, both aquatic and terrestrial use chemical cues or scent to assess the presence of predators, (reviewed in Kats and Dill, 1998). Predator detection by way of stimuli other than visual and mechanosensory is important in aquatic habitats, which are often turbid, rendering visual senses ineffective when assessing the risk of predation. Use

of chemosensory cues to provide effective predator detection has developed in a wide rage of aquatic species. Abrahams and Kattenfeld (1997) demonstrated how turbidity influences behavioural decisions in fathead minnows (*Pimephales promelas*), when in the presence of a predator (yellow perch, *Perca flavescens*). In non-turbid conditions, when the water was clear (so that both predators and prey could see well), they found that the number of fathead minnows in more dangerous habitats were significantly lower when a predator was present. The numbers of minnows in these risky habitats was unchanged in turbid water. This indicates that visual cues are important in the detection of predators by minnows. Despite this change in habitat use, there was no difference in the numbers of prey consumed by yellow perch, highlighting that prey detection by predators is depressed in visually hunting fish when in turbid conditions.

Aquatic invertebrates have especially exploited chemical cues as an early warning system, and many studies have demonstrated how important specific cues can be in the risk assessment and subsequent behavioural modifications in may species. Chemical stimuli from predators can give prey information not just on the presence or absence of a predator, but can give more direct information as to the diet and hunger of individuals, as demonstrated by Åbjörnsson, *et al.* (1997). This study on the aquatic beetle *Acilius sulcatus* shows the ability of this species to detect the level of risk from chemical stimuli emitted from perch (*Perca fluviatilis*). Individuals reduced their activity levels in the presence of chemical cues from hungry perch during both day and night, but not when exposed to cues from satiated perch.

Prey can also utilise chemosensory cues from fish to assess the diet of predators and therefore calculate risk. Chivers, *et al.* (1996) studied the reactions of damselfly larvae (*Enallagma* spp.) to chemical cues from pike (*Esox lucius*) predators fed on different prey items. Naive larval damselflies, which had not previously been exposed to cues from pike, modified their activity depending on which prey item the pike had been fed. With individuals significantly reducing activity in the presence of cues from pike fed damselflies and fat-head minnows (*Pimephales prolmelas*), whilst increasing activity in the presence of cues from those fed mealworms (*Tenebrio molitor*). Prior experience of predatory cues from pike fed damselflies and fat-head minnows led to a decrease in activity when subsequently exposed to pike fed with mealworms.

Van Gool and Ringelberg (1998), studied the influence of a variety of factors on migration in *Daphina*. *Daphnia* spp. migrate to deeper areas of the water column during the day to avoid predation from visual predators, such as juvenile perch and migrate back to the surface a night in order to feed, when there are fewer predators present. Migration was shown to be influenced by both light intensity and concentration of predator kairomones. Predator induced migration was also studied in ovigerous water fleas *Ceriodaphina reticulata* (Seely and Lutnesky, 1998), using different concentrations of exudate from predatory juvenile green sunfish (*Lepomis cyanellus*) at different prey densities. The authors found that migration depth increased with increasing concentrations of green sunfish exudate, but that migration depth was reduced when prey group size increased. This suggested that the density of both predator and prey are important in this species when making decisions to migratē, with high exudate concentrations indicating a high predation risk, which may

be reduced when prey are in high densities. In the presence of predatory cues, individuals decreased their activity, this was not influenced by group size.

Prey can utilise the information gained as to the identity of a predator to modify anti-predator behaviour specifically, to reduce the risk from particular predator species. Turner *et al.* (1999) used both pumpkinseed sunfish (*Lepomis gibbosus*) and several species of crayfish predators to study the anti-predator behaviour in a freshwater snail (*Physella gyrina*). *P. gyrina* are at risk from both these predators throughout their natural habitat, but avoidance involves alternative use of habitats for each predator species. Foraging behaviour in both these predators is very different, pumpkinseed sunfish being unable to forage in covered areas, whereas crayfish inhabit these covered substrates, therefore effective avoidance of these predators involves the use of different habitats. To study this Turner *et al.* used chemical cues from predators as a stimulus, feeding them a measured amount of *P. gyrina*, and keeping the mortality of the prey constant. They found that both predators had different effects on the behaviour of the snails, with fish predators increasing the use of covered substrates, whilst crayfish were associated with a movement of snails out of covered areas to the water surface. This demonstrates that *P. gyrina* are capable of distinguishing between different species of predators from chemical cues alone.

The presence of chemical cues from predators has been shown to induce morphological changes in a variety of aquatic prey species. A study on a similar species of freshwater snail, *Physella virgata virgata* (Crowl and Covitch, 1990) highlighted that predation risk may also effect a species further, and can lead to

changes in life-history strategies. In the presence of odour emitted when the *crayfish Orconectes virilus* fed on *P. virgata virgata* , conspecifics delayed the onset of reproduction, until they had reached a size of around 10mm whereas individuals typically began reproducing at a size of 4mm in the absence of such cues. Predation risk from *O. virilus* is greater when snails are a smaller size, and this risk decreases with increasing size up to around 10mm, when predation from this species becomes uncommon. Therefore it appears that by increasing growth rates to the detriment of reproduction, snails are reducing the risk of predation from this crayfish species.

A similar strategy is seen in the isopod *Lirceus fontinalis*, where different populations have adapted their life history according to the predators present in their habitat (Sparkes, 1996). Females from populations where fish predators were absent, and the main predators are salamander larvae, matured at larger sizes (8-9mm) in order to avoid predation. Salamander larvae are limited to relatively small prey, and by increasing body size, females can reduce the risk of predation. Populations subjected to fish predation by the banded sculpin matured at smaller sizes (6-7.5mm), which left them vulnerable to fish predation. For these populations, increasing body size would not prove effective at reducing predation risk because banded sculpins are not limited to small prey.

Daphnia pulex is an example of a species that can develop morphological changes when in the presence of predatory chemical cues. *D. pulex* are eaten by a wide variety of vertebrate and invertebrate predators. Tollrian (1995b), investigated the morphōlogical changes to *D. pulex* when exposed to chemical cues from the phantom

midge larvae (*Chaeoborus*), which is a predator of this *Daphnia* species. The presence of cues from *Chaeoborus* induced a variety of changes in juvenile *D. pulex*. Individuals exposed to kairomones developed strong neck teeth, which have been demonstrated to be effective in decreasing predation risk from *Chaeoborus* in previous studies (Tollrian, 1995a). Individuals exposed to cues also had a larger body size, which resulted in a higher fecundity, but meant they took longer to mature.

Predation risk can alter population behaviour as well as individual behaviour, as in the case of *Daphnia*, which displayed different levels of anti-predator behaviour when in changing population densities some species seek high population densities as a method of reducing predation (Seely and Lutnesky, 1998). Just as fish that aggregate in shoals gain the advantages of reduced predation risk this can be important for other species. Côte and Jelnikar (1999), found that mussels (*Mytilus edulis*) exposed to water containing cues from a hungry lobster (*Homarus gammarus*), aggregated more readily than in the absence of the cue, suggesting that clumping is used as a method of reducing predation risk.

Boriss *et al.* (1999) suggested a chemical which could be responsible for cues leading to predator identity by invertebrates. They found that small concentrations of the substance trimethylamine (TMA) which is reduced from trimethylamine-N-oxide (TMAO) by bacteria in the skin mucus induced migration of *Daphina hyalina* into deeper water during daylight. However if only low concentrations of TMA were present at night, *Daphnia* migrated back to the water surface. Migration did not occur when TMA concentrations were high. The study goes on to suggest that TMA is only

one of a number of chemicals that can be used by prey species in the identification of predators, because *Daphina* reacted to a greater extent in the presence of water containing fish cues.

Aims

Given these examples of use of chemical cues in predator avoidance in a range of aquatic invertebrates, including crustaceans, and the fact that *A. aquaticus* lives in the benthos, where light levels may well be low, it seemed worthwhile to examine whether this species uses chemical cues in predator avoidance.

Specifically the aim was:

- To determine whether the presence of olfactory cues from potential predatory fish alters shelter seeking in *Asellus aquaticus*.

3.2 Materials and method

Field Site

Asellus aquaticus were collected during February to November 1998 from the Forth and Clyde Canal (at a site near Bishopbriggs, Glasgow), a few days prior to each experimental run. Individuals were caught from within and around macrophytes on the canal bank, using a pond net. They were then transported back to the aquarium in Glasgow, inside watertight containers.

Animal Husbandry

A. aquaticus were kept in a series of stock tanks, in an aquarium maintained at 17°C, with a light regime of 12 hours light, 12 hours dark. Decaying leaves were added to the stock tanks for food, and the tanks were aerated at a slow rate. *A. aquaticus* were sorted according to gender and reproductive status into three different groups:

Males

Brooding females (females carrying broods)

Post-brooding females (females which had released their broods)

The tanks containing precopulatory mate-guarding pairs and brooding females were checked once every day, pairs that were no longer in precopula and females that had released their broods were removed and placed in their appropriate stock tank.

Marking

Three categories of *A. aquaticus* were used in the experiment: males, post-brooding females, brooding females. In order to gather data on individual behaviour, some animals from each of these groups were marked. Marking was carried out using a variety of bright colours of nail varnish, which was 'spotted' onto the dorsal surface of the thorax with a toothpick, whilst individuals were out of water. The marks were left to dry for a short time (up to 3 minutes), before individuals were returned to water, individuals quickly settled down once returned to water, and subsequent survival of marked individuals was not effected (full description of marking procedure in Chapter 2).

Experimental tanks

This series of experiments were carried out during February to May 1998, and November 1998, at the University Field Station, (Rowardennan, Loch Lomondside, Scotland). Experiments were not carried out between June and October due to problems with high temperatures in the experimental tanks.

The experimental set-up consisted of a large fibreglass tank measuring 1.2m X 1.2m, this contained eight smaller tanks which were 340mmX 200mm. These smaller plastic tanks were filled to a depth of 30mm with builders sand. In one end an artificial shelter was placed, this consisted of a plastic coated wire frame with three textile leaves attached. The other end of the tank had two decaying leaves (collected from Loch Lomond) staked into the sand with cut down toothpicks (to prevent

individuals from hiding underneath the leaves). For full diagram of set-up see figure 3.1.

A small pump (Powerhead 402, Aquaclear, Hagen) was used to transfer water from a header tank into the smaller tanks via a system of plastic tubes as a very slow rate so as not to create a current in the tanks. At a height of 110mm above the sand, a series of small drainage holes were bored, to drain away excess water.

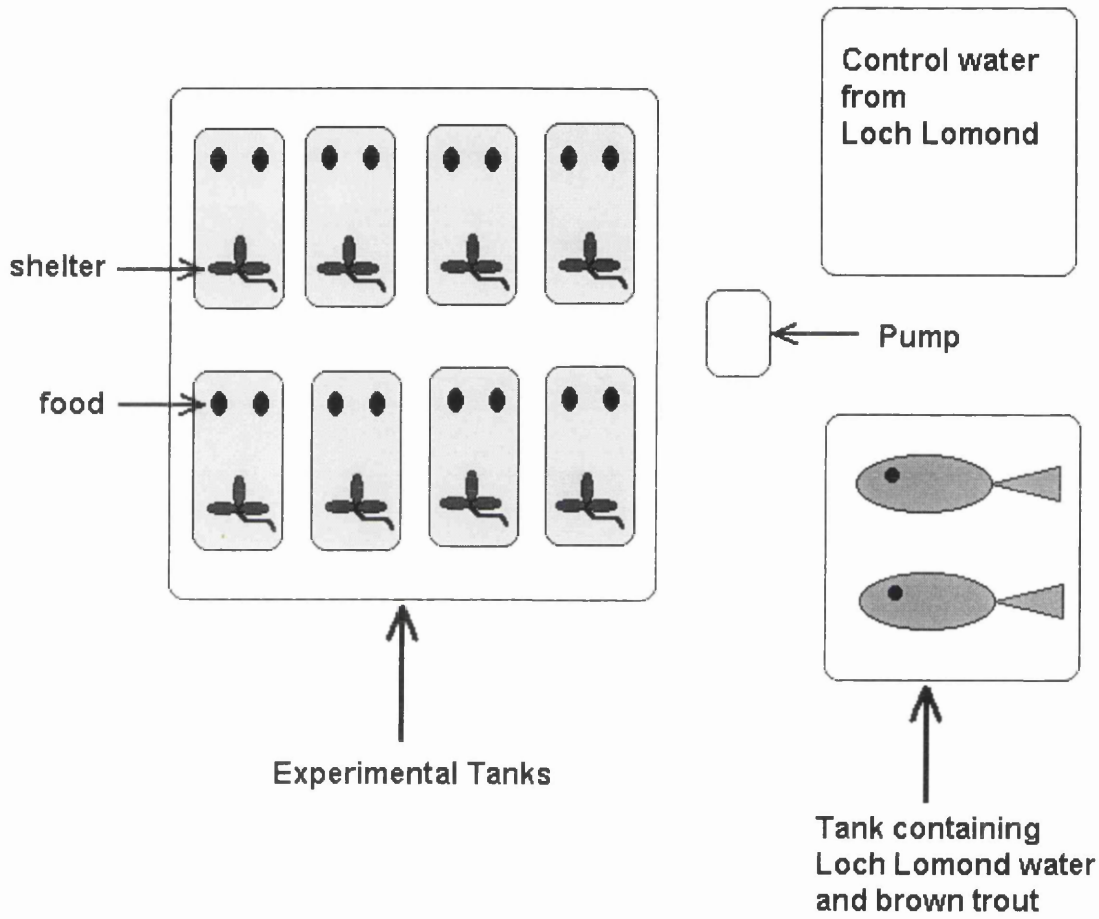
Experimental procedure

Each experimental run was conducted over a period of 2 days and 2 nights. Prior to each experimental run, a day was spent setting up the tanks and manipulating animals. The experimental tanks and sand were cleaned thoroughly before each experimental run, and Milton fluid was used to sterilise the shelters. This cleaning regime was required to prevent any food source other than leaves being present in the tanks. The plastic tubing used to carry water to the experimental tanks was also thoroughly flushed through. Once set up, the experimental tanks were filled with water pumped from a header tank. This had a constant flow of water from the main header tank, which is filled directly from Loch Lomond. From herein this will be referred to as control water.

Ten *A. aquaticus* belonging to the same reproductive category, were introduced to the experimental tanks in the evening and left to settle overnight. On the following day, recordings were made every 30 minutes of both the number of individuals and the

Figure 3.1

Diagram of experimental set-up



position of marked individuals in each tank area, while control water was pumped in. Three different tank areas were recorded:

Shelter (when individuals were in or on the artificial plant).
Feeding (when individuals were observed feeding on the leaves or wooden toothpicks).
Active (individuals are present in any other area of the tank).

In the evening of day 1 when recording ended, the pump was moved to a tank containing several brown trout. This water was pumped into the experimental tanks overnight, and will be referred to as experimental water. Recording was resumed in the morning and carried on over the second day. At the end of day 2, individuals were removed and the experimental equipment was washed to remove any cues left behind by the experimental water.

It was not possible to randomise the sequence by using water containing cues on the first day, following this with control water, because there would be a possiblilty that cues would persist within the tanks between days. It was not possible to clean tanks between both days of the trials, as it would disturb the individuals. Therefore a different control regime was used.

In order to ascertain whether changes in behavioural patterns could be attributed to chemical cues, or whether this was simply due to temporal changes in habitat use over

the two day experimental period, a control run with eight repetitions was conducted, using control water on two consecutive days.

Attempts were made to complete the same number of repetitions for all groups, however there were problems with obtaining some of the groups, due to the fluctuating population structure and reproductive cycle in this species throughout the year. Females that had not mated were difficult to find in the initial experimental period, and all females were difficult to find in the November run, due to many dying as a result of reproduction. Mate-guarding pairs were difficult to find outside of April to September, and due to the a combination of the marking process and the length of the experiment it was difficult for pairs to remain together over each experimental run, therefore these were not generally used in the experiments.

The repetitions are as follows:

Group	**Repetitions**
Males	19
Brooding females	5
Post-brooding females	5
TOTAL	29

Data Analysis

It is possible that the behaviour of *A. aquaticus* is different during the night, and therefore to prevent changing day length throughout the duration of these experiments influencing any results, the data used in all analyses were taken from 10:00 to 14:00.

Data were collected on both the proportion of time individual *A. aquaticus* spent in each activity, and the percentage of individuals within each group of *A. aquaticus* that were observed in each activity. These data were analysed using a Wilcoxon matched pairs (signed ranks) test. Data analysis was carried out using SPSS version 8, Minitab version 11 and Microsoft Excel version 7.

3.3 Results

Experimental runs: Group data

Because data had been collected on both group and individual behaviour, it was possible to analyse these separately. Group data used referred to the median number of individuals in each tank area over an experimental run. Individual data referred to the proportion of time each individual spent in the different tank areas over each experimental run (based on 1 observation every 30 minutes).

Wilcoxon matched-pairs signed-ranks tests were used, to directly compare the median proportion of *A. aquaticus* in each tank area on both the initial control day (water containing no cues) and subsequent experimental day (water with cues). The purpose of which, was to see if the presence of chemical cues from fish was associated with a change in behavioural patterns.

I found that significant differences existed in the behaviour between these two treatments when all categories of *A. aquaticus* were combined, see table 3.1 for statistics, and figure 3.2a. The results indicate, that females and brooding females have little variation in their behaviour under both treatments, see figure 3.3a. This may be due either to the tendency that brooding females have for high levels of shelter use in the absence of any predatory threat (personal observation), but could also be due to small sample sizes. Males were seen to alter their behaviour when in the presence of chemical cues, increasing shelter use whilst decreasing activity. They

Table 3.1

Group data, median proportion of individuals per activity experimental run

Category	Activity	N	Z	P
All	feeding	30	-1.98	0.048
All	shelter	30	-3.07	0.002
All	active	30	-3.29	0.001
Males	feeding	19	-2.11	0.035
Males	shelter	19	-2.52	0.012
Males	active	19	-2.79	0.005
Females released-broods	feeding	5	0.00	1.00
Females released-broods	shelter	5	-1.46	0.14
Females released-broods	active	5	-1.29	0.20
Brooding females	feeding	5	-1.00	0.32
Brooding females	shelter	5	-1.46	0.14
Brooding females	active	5	-1.46	0.14

Table 3.2

Individual data, proporiton of time each individual spends per activity experimental run

Category	Activity	N	Z	P
All	feeding	129	-0.31	0.755
All	shelter	129	-3.26	0.001
All	active	129	-3.54	<0.001
Males	feeding	47	-0.31	0.753
Males	shelter	47	-2.54	0.011
Males	active	47	-2.70	0.007
Females released-broods	feeding	54	-0.13	0.897
Females released-broods	shelter	54	-1.41	0.159
Females released-broods	active	54	-1.62	0.105
Brooding females	feeding	28	-0.95	0.343
Brooding females	shelter	28	-1.67	0.095
Brooding females	active	28	-1.71	0.087

Figure 3.2a

Changing behavioural patterns during experimental runs, using median proportion of individuals for each activity

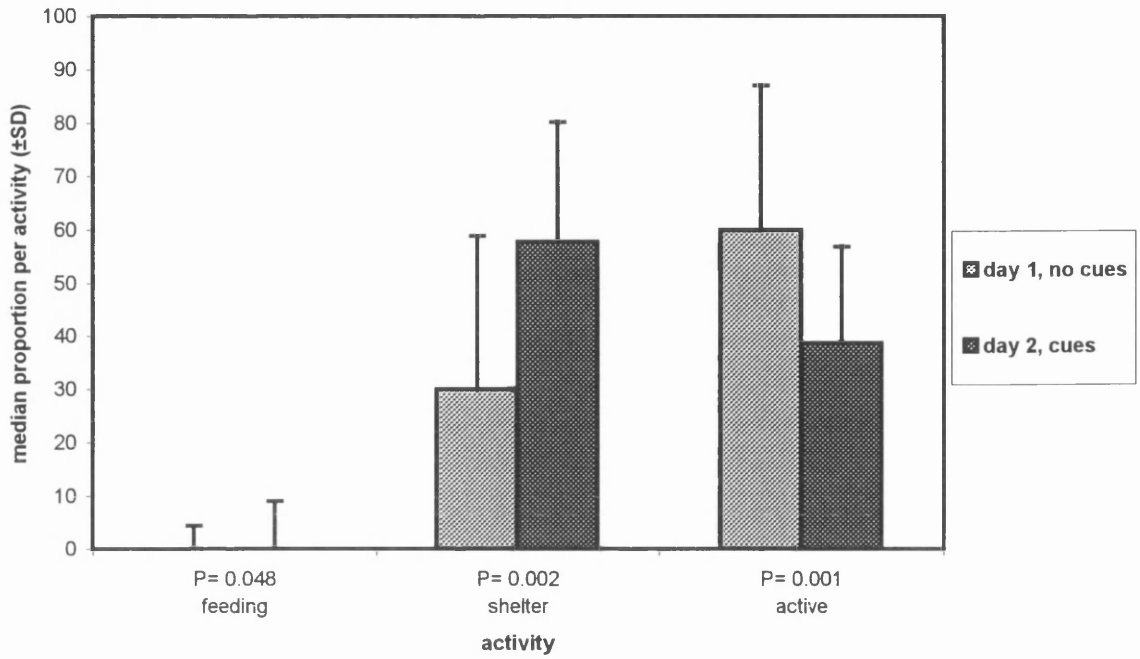


Figure 3.2b

Changing behavioural patterns during control runs, using median proportion of individuals for each activity

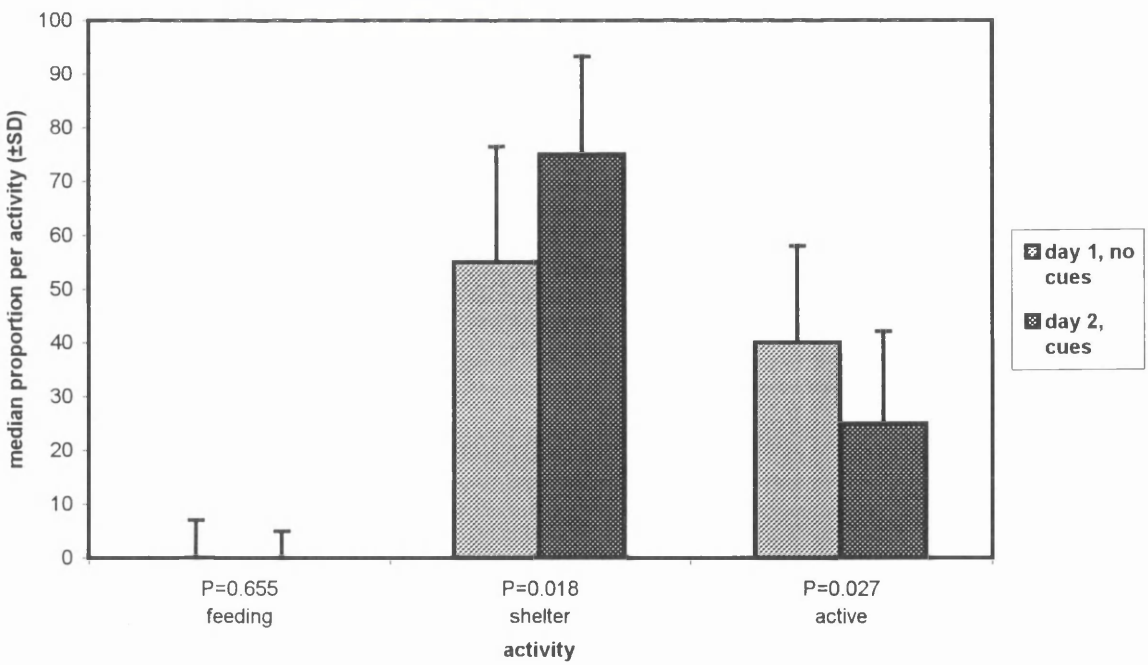


Figure 3.3a

Behaviour of *A. aquaticus* reproductive categories in the presence and absence of chemical cues from predatory fish

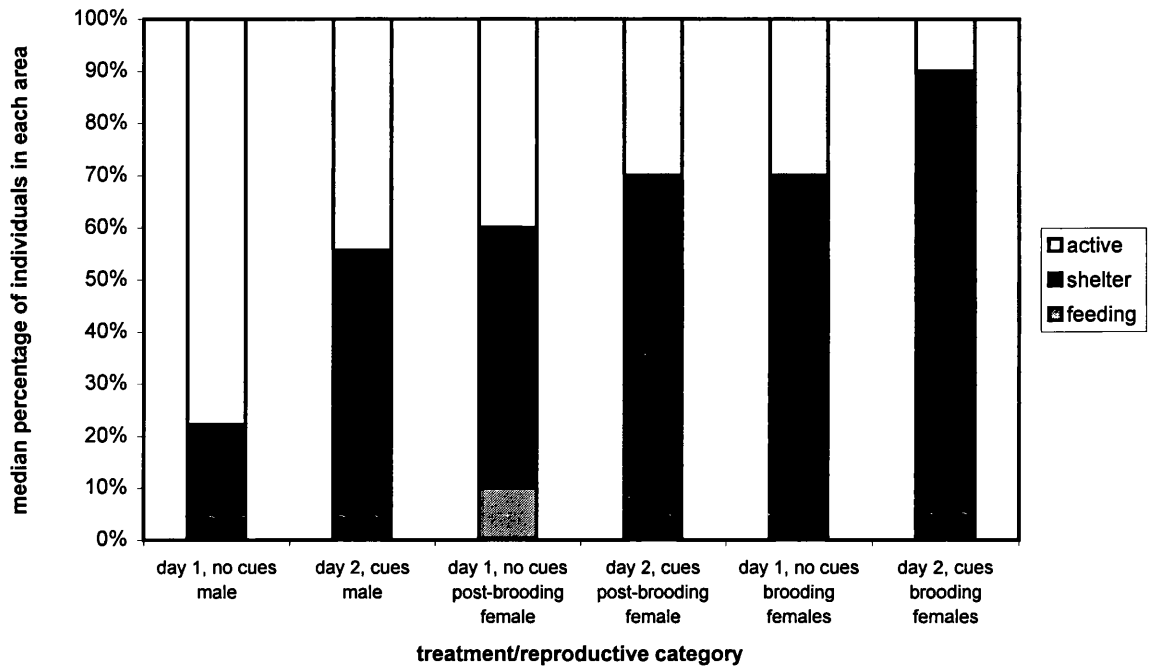
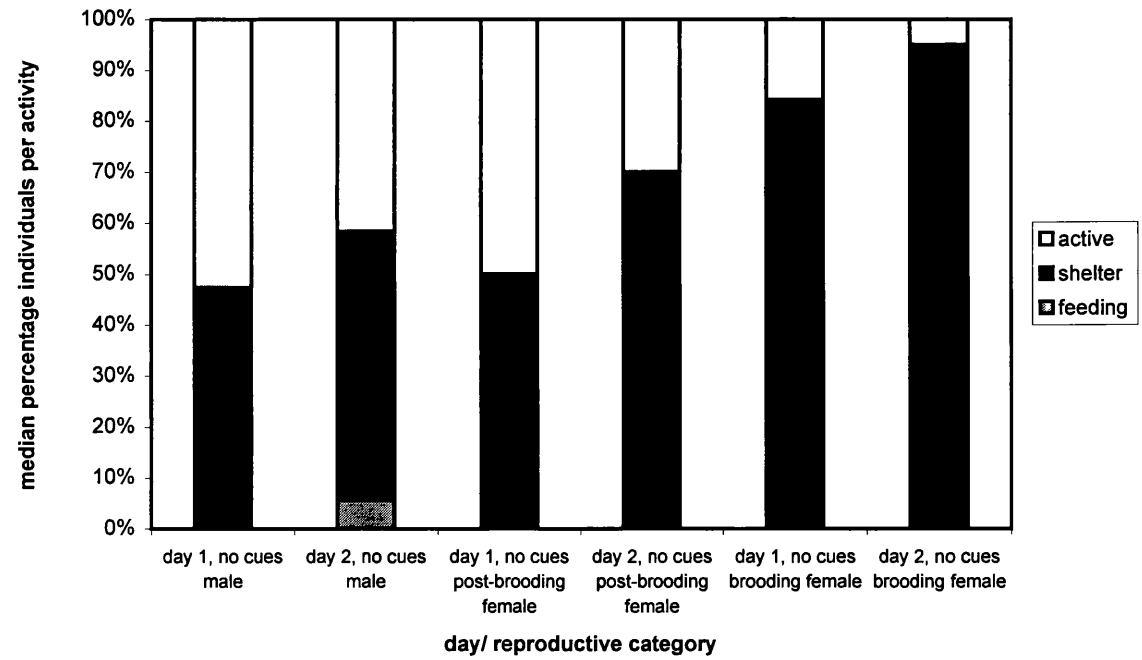


Figure 3.3b

The changing behaviour of different *A. aquaticus* reproductive categories over two successive control days



also show a surprising trend of feeding more, which may be a result of being in the experimental tank for longer, and therefore have elevated hunger.

These trends can be seen in figure 3.3a, which also shows the high levels of variance in activity and shelter use between groups, although this variance decreases in shelter use and activity in the presence of cues.

Experimental runs: marked individuals

Data on the proportion of time spent by marked individuals in each tank area under both treatments was also examined, to see if the presence of predatory cues influenced individual behaviour. The same trends were seen in this data set as in the group data, with shelter use increasing and activity decreasing, see table 3.2 for statistics, and figure 3.4a . The data with all categories combined shows that the proportion of time individuals spend in shelter increases, while activity decreases. Feeding, however, did not change, suggesting that individual that are feeding tend to carry on regardless of risk. Irrespective of reproductive status, females showed little change in their behaviour during the experiment (see figure 3.5a). I attributed this to the increased levels of shelter use in these individuals during the control day.

Control runs

Group data

Due to the time constraints during this experiment, only eight repetitions of controls were carried out. This did not give us sufficient group data to examine categories separately under statistical analysis, however the data are represented in figure 3.3b.

Figure 3.4a

Changing behavioural patterns during experimental runs, using the median proportion of time spent by individuals in each activity

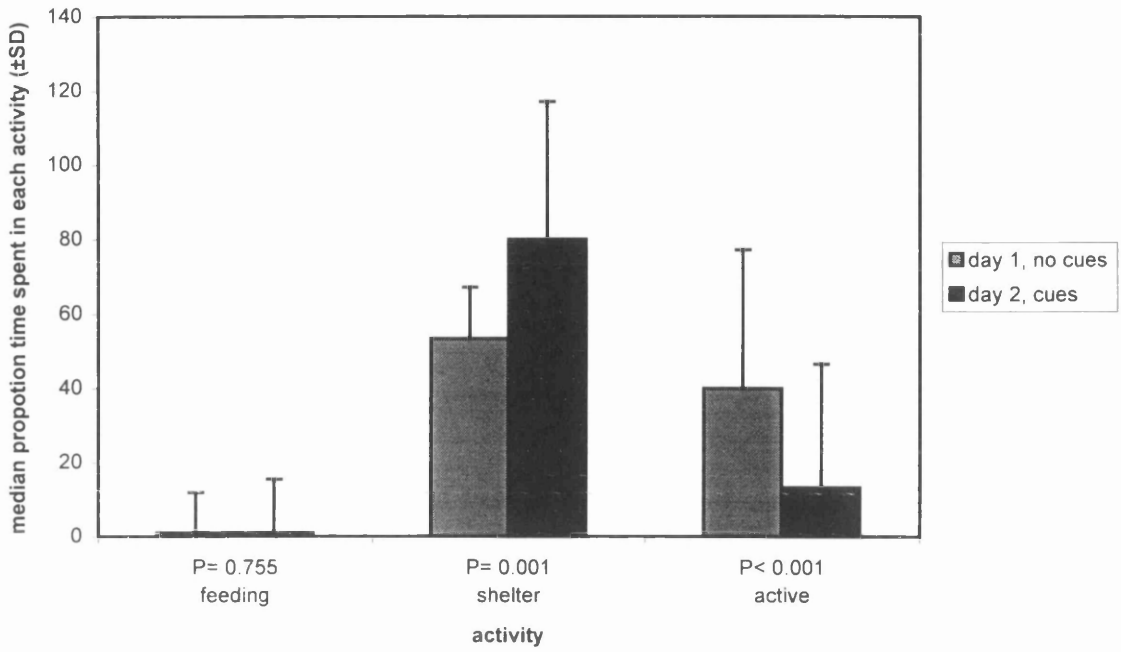


Figure 3.4b

Changing behavioural patterns during control runs, using the median proporiton of time spent by individuals in each activity

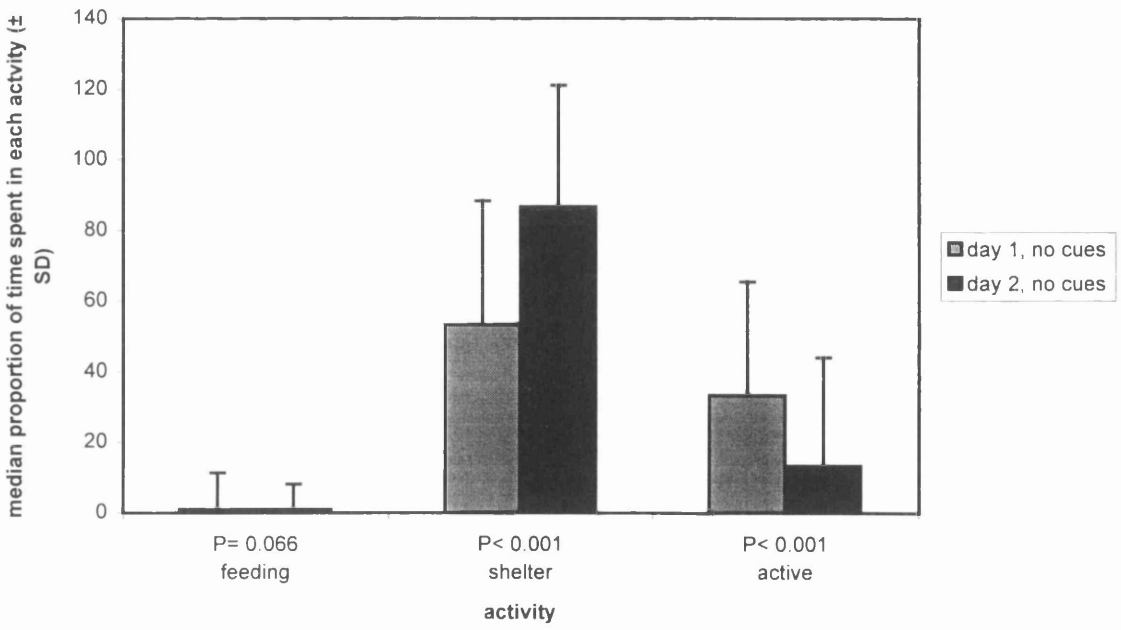


Figure 3.5a

Changing proportion of time devoted to each activity in the presence and absence of predator cues, across different categories of *A. aquaticus*

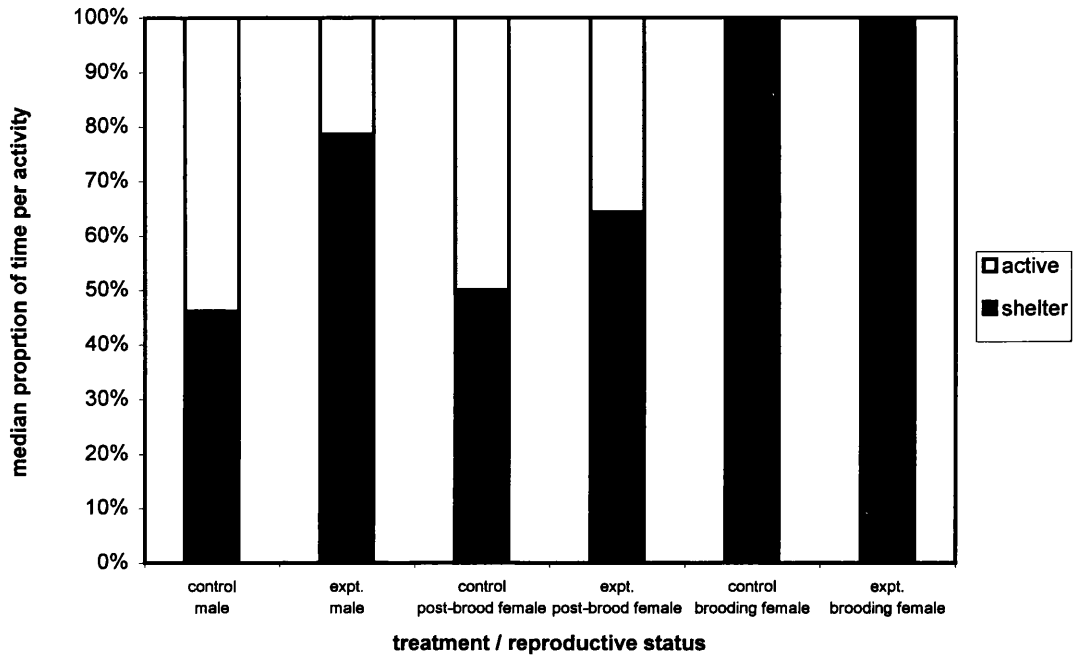
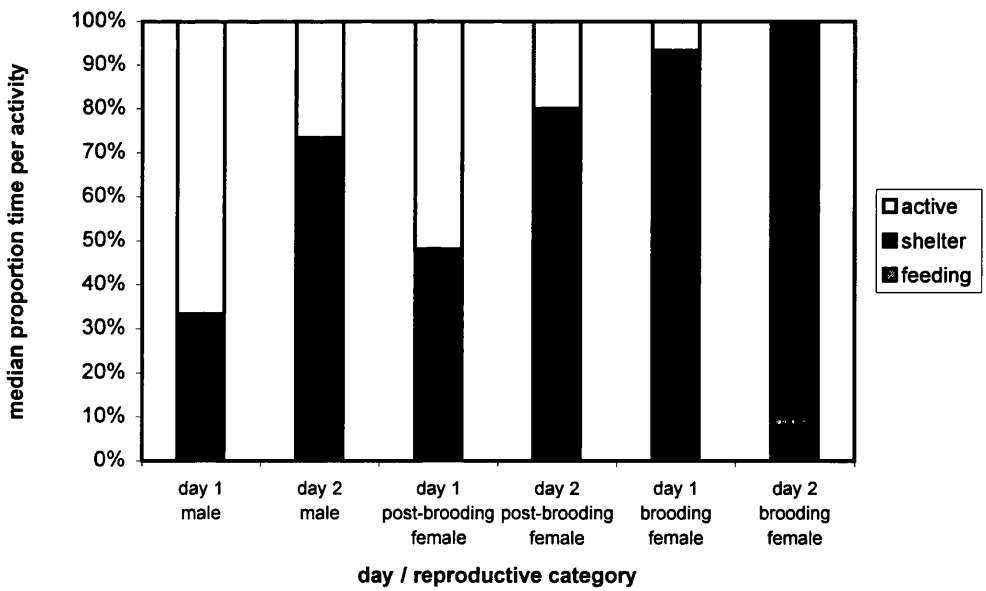


Figure 3.5b

Changing proportion of time devoted to each activity during 2 control days across different categories of *A. aquaticus*



During this period, shelter use was very high, (even on day 1), see figure 3.2b. I found significant differences in the median proportion of *A. aquaticus* in shelter (n = 8, z = -2.375, P = 0.018), and active (n = 8, z = -2.207, P = 0.027), although no differences were found in feeding (n = 8, z = -0.447, P = 0.655) see figure 3.2b. These differences were not as great as those found when comparing behaviour in the presence and absence of predatory cues. However this prevents me from attributing this behavioural change entirely to the presence of chemical cues. The variance that exists in this decreases less on control day 2 than on the experimental day 2, which suggests that the behavioural change is less marked in these control runs then the experimental runs.

Individual data

When making the same comparisons with data on the proportion of time individuals spend in each of the different areas, we can separate out the categories more effectively than with the group data, as there are more data points. The data in Table 3.3 suggests some different trends to the group data, with differences occurring with respect to shelter and activity across all categories, which can be seen in figure 3.4b, which shows these differences with the median for each individual combined. Figure 3.5b shows the extent of the changes in microhabitat preference across both control days. These differences in our control suggests that individuals may show a greater response to temporal effects throughout the course of the experiments than any reaction to chemical cues from predators, although we cannot completely rule out influences from chemical cues.

Table 3.3

Individual data, proporiton of time each individual spends per activity control run

Category	Activity	N	Z	P
All	feeding	62	-1.84	0.066
All	shelter	62	-3.55	<0.001
All	active	62	-3.61	<0.001
Males	feeding	19	-0.12	0.905
Males	shelter	19	-2.17	0.03
Males	active	19	-2.44	0.015
Females released-broods	feeding	30	-1.64	0.102
Females released-broods	shelter	30	-2.23	0.025
Females released-broods	active	30	-2.17	0.030
Brooding females	feeding	13	-1.63	0.102
Brooding females	shelter	13	-2.39	0.017
Brooding females	active	13	-2.39	0.017

3.4 Discussion

Responses of prey to chemical cues from potential predators

In the experimental runs, there were behavioural differences between the first and second day, especially for males, with more shelter use and decreased activity, in the presence of chemical cues from trout. This was associated with an increase in feeding activity. However, a similar change between days 1 and 2 of the trial was observed in the control condition. This suggests that the change observed in the experimental run may have been due to temporal trends, regardless of the presence or absence of chemical cues, although the change in shelter use and activity was more marked in individuals subjected to chemical cues than in the control. It was surprising that the use of shelter differed significantly between both days in the control runs, as previous experiments conducted with a similar design (chapter 2) found no differences in shelter seeking between trials, conducted on successive days.

When separated out according to gender and reproductive status, only males were significantly altering their behaviour in the presence of chemical cues. Males had a much lower level of shelter use and higher levels of activity than both brooding and post-brooding females. This supports previous studies on gender and reproductive status specific behavioural patterns in this species (chapter 2).

Responses to specific cues

There are several reasons that may account for the lack of difference between experimental and control trials. It is possible that *A. aquaticus* only react strongly to

predatory cues from hungry predators, or from predators that have previously fed on conspecifics. The predator used in these trials was fed on pellets, and remained satiated during the experiments. Studies on some species have highlighted the importance of cues specific to hungry predators. This phenomenon is seen in the water beetle *Ascilius suculatus*, which exhibited antipredator responses in the presence of chemical cues from starved perch (*Perca fluviatilis*), but not when exposed to cues from satiated perch (Åbjörnsson *et al.*, 1997). Visibility also proved to be an important factor in anti-predator responses in this species, as when the experiment was repeated during daylight, activity levels did not decrease in the presence of cues from hungry perch. This suggests that *A. suculatus* use a combination of visual and chemical cues to assess predation risk, but that when visual cues are not effective they rely on chemosensory stimuli to detect predators. In my experiment, the behaviour of *A. aquaticus* was only studied during daylight hours. It is therefore possible that chemical cues from predators may have less impact, when visual cues confirm that no predators are present. It has been suggested from previous studies on *A. aquaticus* behaviour, that water disturbances are important in eliciting escape responses (Lyndon, 1996). Possibly a combination of chemosensory, mechanical and visual cues is important in assessing predation risk during daylight hours, with non-visual stimuli becoming more important at night.

The responses of prey to chemical cues can be highly specific to predators that they are at a direct risk from. *Gammarus minus* displayed anti-predator responses to the predatory fish, bluegills (*Lepomis macrochirus*) and striped shiners (*Luxilus chrysocephalus*), which actively preyed upon this amphipod (Wooster, 1998).

However, no anti-predator responses were elicited by the presence of the salamander *Eurycea bislineata*, which is known to be a predator of amphipods, although did not appear to feed on them in this experiment, suggesting that *G. minus* only reacts to chemical cues from predators fed with conspecifics.

It is therefore possible that *A. aquaticus* only react to cues from predators that have eaten conspecifics. A previous study investigated the use of chemical cues in interactions between *Gammarus pulex* and *A. aquaticus*, (Bengtsson, 1982). *G. pulex* are known predators of *A. aquaticus*, who they also compete with for fungal food sources. Bengtsson used an artificial stream to study the behaviour of both species when downstream of either *A. aquaticus* or *G. pulex*. When *A. aquaticus* were placed upstream of *G. pulex*, the predatory *G. pulex* were attracted to the prey. When *G. pulex* alone were placed upstream of *A. aquaticus*, a low level of predator avoidance was seen. However a greater anti-predator response was observed in downstream *A. aquaticus*, when additional individuals were placed upstream of *G. pulex*. This suggests that *G. pulex* are attracted to chemical cues from *A. aquaticus*, which induce them to secrete cues that can be utilised by *A. aquaticus* in predator avoidance. This response is specific to interactions involving both *A. aquaticus* and *G. pulex*.

Predator avoidance by the use of chemical cues has been observed in other isopod species, with respect to cues from fish predators. A study on the isopod *Lirceus fontinalis*, investigated the use of macroalgal (*Cladophora*) shelter by isopods in both the presence and absence of foraging green sunfish (*Lepomis cyanellus*), and their chemical cues (Holomuzki and Short, 1988). They found that in the presence of fish

predators, individuals already present in *Cladophora* remained there, and those that were not in shelter became less active, (whilst remaining outside of the shelter). The same trend was seen in the treatments with water containing chemcial cues from green sunfish, demonstrating that *L. fontinalis* can detect the presence of predators from chemical cues alone. Both these examples (Bengtsson, 1982; Holomuzki and Short, 1988) suggest that the *A. aquaticus* do respond to chemical cues from predators, but that these cues may be specific either to predators that they have prior experience of, predators that pose a direct threat, namely those which contain cues from the predators diet, or from cues released by injured conspecifics.

Persistence of cues in the aquatic environment

An additional factor that may influence the response of prey to chemical stimuli from predators is the gradient of cues, and their persistence in the aquatic environment. Because anti-predator responses, mediated by chemical cues from predators, can be costly in terms of reduced food intake that may also have implications for fecundity (Holomuzki and Short, 1988; Peckarsky and McIntosh, 1988), it is important that prey species can assess the level of risk, and adjust their behaviour accordingly. Kats and Dill (1998) review two studies where prey continued to respond to cues from predators for several days after the removal of the predators. Therefore the concentration of cues present may be important in assessing the extent of predation risk. A study on the isopod *Lirceus fontinalis* suggested that the reaction to chemical cues from predators is relatively short term (Holomuzki and Hatchett, 1994). Individuals stopped responding to cues up to three days after their introduction. It is possible that the *A. aquaticus* in my study reacted more strongly to cues following

their initial introduction, which occurred overnight, when behavioural data were not collected.

Improvements to the experimental design

There are also various factors within the design and execution of the experiment that may have resulted in the absence of detectable influence of chemical cues. If repeating this experiment, I would make several changes to the experimental design and protocol. During the course of the experiment, there were problems with high temperatures in the experimental tanks, and therefore the study was halted for several months over the summer and resumed again in the autumn. All control runs were carried out during the spring and summer, whereas the experimental runs were conducted in the autumn as well. This may have caused several problems, particularly due to the difference in reproductive behaviour over this period. Photoperiod could also have influenced the data, although I adjusted for this within the trials by only using data collected between 10am and 3pm. Therefore the experiments would be repeated in a temperature and lighting controlled aquarium with no external windows.

I also realise the importance of running both experimental and control trials at the same time, to reduce the influence of external factors, and to collect the same number of repetitions for both conditions. This was not possible at the time of these experiments, because space constraints limited me to using just one tank. If repeating these experiments I would use two separate tanks running together, so that control and experimental runs could be carried out simultaneously.

I would also make changes to the predators used in the experiment. Using a species which they would have gained experience from in their natural habitat. Perch are known predators of *A. aquaticus* in the Forth and Clyde Canal. If repeating the experiment, I would use cues from starved perch that had been fed previously on *A. aquaticus*. This would pose a more realistic threat to the prey species. It was not possible to use this set up originally, due to lack of tank space in which to keep the additional fish I required, combined with a serious disease problem that limited the movements of fish both into and out of the field station.

Conclusions

We were unable to ascertain whether behavioural decisions in *A. aquaticus* were influenced by chemical cues from potential predators, as our control runs displayed the same trends as our experimental runs. Both showed the trend for groups and individuals to increase shelter use and decrease activity on the second day of the experimental run, regardless of the treatment.

From this we cannot conclude that *A. aquaticus* do not utilise chemosensory cues in predator avoidance, as in our experiment these cues would have been very dilute. It is possible that *A. aquaticus* respond to cues when predators can be detected by other senses, such as disturbance.

Chapter 4

Foraging behaviour of ruffe (*Gymnocephalus cernuus*) for concealed prey in the absence of visual cues

4.1 Introduction

Sensory control of foraging in fish

Extensive work has been carried out into foraging in fish. The majority of this work has been centred on fishes ability to forage visually (Abrahams and Kattenfeld, 1997; Gibson, 1983; Peterson and Gadomski, 1994; Savino and Stein, 1989; Warburton, 1990). It is now accepted that many other senses are important in searching for and acquiring food, such as chemoreception, electroreception and mechanoreception. The ability to use senses other than sight is important for many species, as they may live in environments or have an activity patterns which limits them to foraging under conditions of little of no light, in turbid environments.

Because of the problems associated with making behavioural observations in the dark, most studies either allow visual cues or use some highly invasive technique (like removing eyes), even if they have addressed non-visual cues.

Chemoreception

In spite of the concentration of work on visual cues, numerous studies have highlighted the importance of non-visual senses to foraging fish. Chemical stimuli can allow an individual to gain much information about its surrounding environment (Carr, 1988; Hara, 1993). Chemical cues are known to be highly important to many species of aquatic animals. Chemical signals can provide information about a variety of factors such as other individuals within social groups, potential mates, presence of predators and location of prey (Atema, 1980; Atema *et al.*, 1980). Chemical stimuli

are important in some species for the search, location and capture of prey. When a fish detects food via chemical cues this leads to the initiation of searching behaviour, which in many species can be identified as three stages. Initially fish exhibit excitement, followed by a period of searching to locate food items; the final stage is food ingestion or prey capture (Jones, 1992). Some species such as the Ictalurid catfish (*Ictalurus natalis*) have special adaptations to assist with the location of prey by chemical stimuli (Atema, 1971; Bardach *et al.*, 1967). Once an individual has detected prey via chemical cues it will begin to use maxillary barbels. The same is true of the black bullhead (*Ictalurus melas*) (Olmsted, 1918). Some species which do not possess such appendages will go on to use other senses once stimulated to forage, such as flounders (Stutterlin, 1975), which go on to feel the surface substrate with their bodies.

Electroreception

Some species of fish are able to employ electroreception in detecting prey. This enables fish to detect prey in dark and turbid waters, or prey that is concealed from view, possibly buried in a substrate (Kalmijn, 1988). Different methods are employed by different species. Some fish species can generate weakly electric fields, such as *Gymnarchus*, which detect conductors and non-conductors present in its electric field (Lissmann, 1958). Elasmobranchs are able to detect bioelectric fields of other animals. Dogfish (*Scyliorhinus caninula*) were unable to detect the presence of a flounder when buried in a chamber covered with insulating plastic film (Kalmijn, 1971), but could when the flounder was buried in a chamber of agar, which has good conductivity. The elephant nose fish (*Gnathonemus petersii*) posses an appendage

resembling an elephants trunk, which is covered with electroreceptors, making then particularly sensitive to the electric fields generated by other species (Bell, 1986; Montgomery, 1991).

Mechanoreception

All teleost fish posses a lateral line, which consists of pores leading to a network of sensory canals (Bleckmann, 1993). Some species of fish have more highly developed lateral lines, which are much more sensitive, enabling them to use their lateral lines for foraging, by employing mechanoreception whilst searching for and catching prey. This is true of many species of deep-sea fish, and other species which forage in the dark, such as the torrentfish (*Chemiarrichthys fosteri*) (Montgomery and Milton, 1993). Alewives, (*Alosa pseudoharengus*) are able to use their lateral lines to feed particulately on many species of zooplankton at night (Janssen *et al.*, 1995) exploiting the diurnal migration of zooplankton into shallower waters at night (Lazzaro, 1987; Young and Watt, 1993). It was observed that foraging alewives adopted altered swimming patterns similar to those displayed by the blind cave tetra (*Astyanax jordani*), when foraging (Abdel-Latif *et al.*, 1990), probably to reduce hydromechanical signals generated by their own swimming pattern. The mottled sculpin (*Cottus bairdi*) also employs the lateral line in detecting prey (Hoekstra and Janssen, 1985) as do the other sculpin species *Batrachocottus baicalensis* and *Paracottus kneri* (Janssen *et al.*, 1999). However instead of modifying swimming behaviour, they remain stationary on the lake bottom in order to detect movements by prey. This behaviour is slightly modified in streams, where they use distortions in laminar flow to locate prey (Hoekstra and Janssen, 1985). A study of four species of

notothenid fish (Janssen, 1996), showed how well specialised lateral lines can be. It found that benthivores had a higher sensitivity to prey along the whole body, whereas the two planktivores studied only displayed receptivity to hydromechanical cues at the head.

The importance of using a combination of senses

Most animals tend to use one sense predominantly over others; however it is important for most species to use a combination of different senses in order to forage effectively and efficiently under fluctuating environmental conditions. The Chinese perch (*Siniperca chuatsi*) is an example of such a species, which can use senses independently of each other, but that functions better when able to utilise a number of distinct stimuli (Liang *et al.*, 1998). The foraging ability of Chinese perch was examined using several different stimuli whilst selectively blocking others, in order to ascertain the importance of each sense in foraging. Foraging was not adversely effected when just one sense organ was blocked (be it vision, lateral lines or olfaction). Individuals relying entirely on visual or mechanosensory stimuli also foraged effectively, although when relying solely on the lateral line to detect prey, they were unable to distinguish moving prey from vibrating objects, attacking both. This behaviour was not observed when visual cues were available. When relying entirely on olfactory cues, very few prey items were taken. However olfactory stimuli were important in distinguishing live prey from dead or artificial prey once caught. These findings correlate well with the behaviour of Chinese perch in the wild, these fish feed exclusively on fish, stalking them before attacking. In the wild, Chinese perch occupy habitats that are subject to high levels of seasonal turbidity, this

combined with the nocturnal feeding behaviour exhibited by this species, demonstrates the importance of non-visual senses in foraging.

Another example of the different relative importance of senses in the acquisition of food can be found in *Gnathonemus petersii*, an electric fish (Von der Emde and Bleckmann, 1998). The ability of fish to forage on living and dead chironomid larvae were compared under a variety of conditions, whilst some fish had their electric organ rendered ineffective through surgery, others remained intact. Most individuals that were unable to utilise active electrolocation could still forage effectively in the dark. The study demonstrated that *G. petersii* can utilise a combination of active and passive electrolocation, vision, olfaction and the lateral-line in order to forage, but that the extent to which each sense is used is dependant on each individual, for example some rely on electrolocation more than others.

Most species have a sense that they utilise more than others. For example perch, (Bergman, 1988) rely mainly on vision. In some species one sense will override others, when stimuli are detected. This is seen in the green sunfish (*Lepomis cyanellus*) and the largemouth bass (*Micropterus salmoides*), (Janssen and Corcoran, 1993). When presented with prey in a feeding tube, bites towards the prey were elicited by all the fish. However when a jet of water was squirted, this drew the attention of the fish away from the food source and towards the jet (a mechanosensory stimulus). This indicates that although vision is important for both these species, the presence of strong mechanosensory cues can override visual stimuli when foraging.

Both species forage at dawn and dusk on cryptic prey, and therefore the use of non-visual senses are essential.

Foraging studies in ruffe and perch

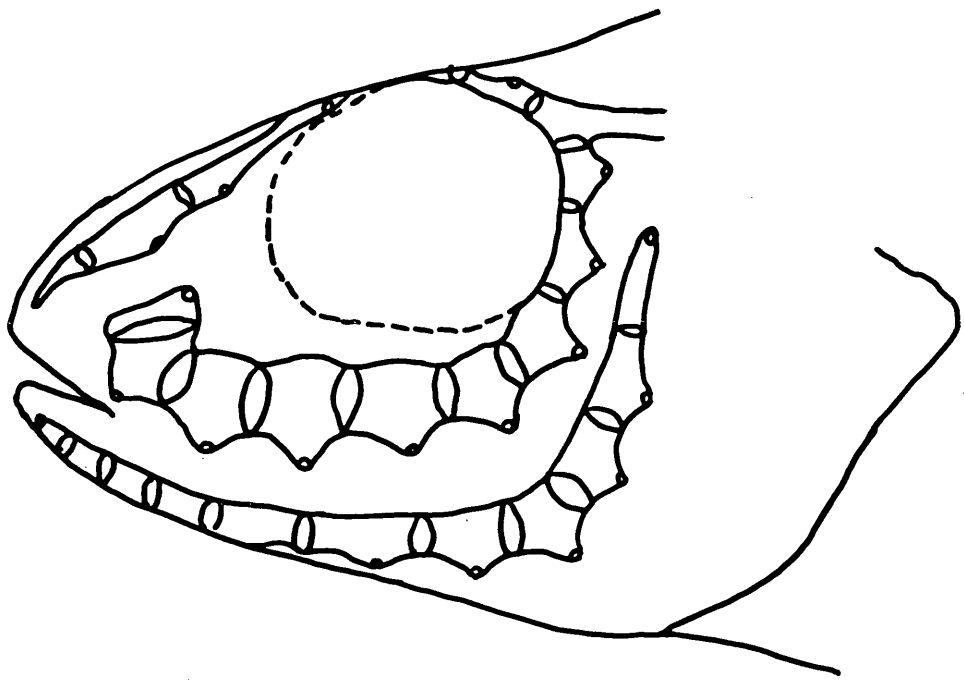
Ruffe (*Gymnocephalus cernuus*) are benthivorous fish, adapted to feeding on small benthic invertebrates under conditions of low light in turbid environments, therefore any adaptation to enable ruffe to feed under such conditions would be advantageous. A morphological feature of ruffe, which is also seen in many deep-sea fish, is the highly developed lateral line system (see figure 4.0 for diagram of ruffe lateral line). This is unusual in freshwater fish (Disler and Smirnov, 1977; Jakubowski, 1963; Jakubowski, 1967; Wubbels, 1991), and it has been shown that the canals surrounding the head in ruffe, are sensitive enough to locate and successfully attack prey in the absence of visual cues (Gray and Best, 1989).

There are relatively few studies on the foraging behaviour of ruffe, and those that do exist have been carried out from the perspective of investigating the competitive abilities of ruffe against other fish species such as perch (*Perca fluviatilis*, Bergman, 1987; Bergman 1988). These studies do provide us with useful information on the foraging behaviour of ruffe, and their ability to forage under different environmental conditions. An investigation was made into the influence of light intensity on the foraging abilities of ruffe and perch on two different prey species, *Daphnia magna* and *Chaoborus obscuripes* (Bergman, 1988). Bergman found that perch had significantly higher capture rates and attack success than ruffe at high light intensities (10 and 1 lux), whereas no differences were found between the two at 0.02 lux.

Figure 4.0

Diagram of a ruffe head showing the lateral line canals

(After Jackubowski, 1963)



In total darkness, ruffe were found to have significantly higher capture rate and attack success than perch. Behavioural observations showed that ruffe spent more time close to the bottom of the tank, whereas perch foraged in the water column. Both fish displayed the same trend of having higher capture rates with higher light intensities, although the attack success of perch decreased with decreasing light intensities, the attack success of ruffe remained unchanged. These differences in behaviour with different light intensities indicates that ruffe are using other senses in order to locate prey, such as the lateral line. This could help ruffe locate prey in darkness, by detecting their movements enabling them to forage effectively at night, in turbid water and at great depths. A related study investigated the influence of temperature on the foraging behaviour and swimming abilities of these two fish species (Bergman, 1987). It was found that at temperatures of 16 to 20°C, perch had a higher swimming performance than ruffe, although the converse was observed at 4 and 8 °C. Perch were also found to be more successful predators than ruffe, although the differences in attack success between the two decreased with decreasing temperature. This indicates that ruffe are better adapted to a wider range of temperatures than perch. Bergman classified ruffe as "temperature generalists" and perch as "temperature specialists". It was also observed that in the light perch had a longer reaction distance to prey than ruffe, indicating that they are more visually motivated. Both these studies relate to the distribution of ruffe and perch in the wild, where perch are absent from the cooler, darker areas (Bergman, 1987; Bergman, 1988).

A previous study examined the changing foraging behaviour of developing ruffe and perch fry (Disler and Smirnov, 1977). They observed that early on in development

perch and ruffe larvae rely on vision for food acquisition and large water vibrations to signal danger. The lateral line begins to play a more important role in prey detection in both species near the end of the larval stage. In the fry stage mechanosensory stimuli become more important and the two species begin to display several differences in both behaviour and morphology, with ruffe foraging near the bottom, on their own and perch actively foraging in schools. At this stage, perch are seen to rely more on vision than ruffe, whereas ruffe begin to use their lateral lines more.

Aims

There have been few detailed studies on the behaviour of ruffe when foraging in the dark, from this investigation I hope to learn more about the ability of ruffe to locate prey in the absence of visual cues, and to search for prey buried in a substrate. Various aspects of defined behaviours will be analysed, to give an insight into whether ruffe can distinguish between pots with food and without food simply by using non-visual cues, or whether they can learn over the course of an experiment.

- To ascertain whether ruffe forage effectively in the absence of visual cues.

- To describe the behaviour of ruffe when searching for prey in the dark.

- To investigate the ability of ruffe to forage for buried prey items within a substrate.

- To discover whether ruffe can distinguish between pots containing prey and those without.

4.2 Materials and Method

These experiments were carried out at the University of Glasgow between March and December 1998. The ruffe (*Gymnocephalus cernuus*) used in these experiments were caught from Loch Lomond at various dates using both fyke nets and a bottom trawl. The experiments were conducted within a rectangular glass tank measuring 1.2m X 0.60m with a depth of 0.38m, which was divided into two separate areas by a partition. The partition was made of white foamex, and consisted of a door 0.46m wide and 41cm high, which could be lifted out from above. This partition was fitted closely to the tank sides and held in place by supports, which were glued to the tank sides. The sides of the tank were covered on the inside by white sheets of foamex, to stop internal reflections in the tank influencing fish behaviour. The living area of the tank measured 0.40m X 0.60m, and contained a shelter and plastic aquarium weed, the experimental area measured 0.80m X 0.60m and contained nine clear plastic pots with a diameter of 62mm and a depth of 32mm. The tanks were filled with coral sand to a depth of approximately 30mm, to provide a substrate to bury the pots into and a pale background against which the fish could be distinguished more easily. The original idea was to conceal the pots in the tank substrate; however this would have a required a depth of gravel that would have caused tank hygiene problems, so pots were only partly submerged amongst the gravel (although prey were completely hidden).

A frame above the tank held a closed circuit television camera, which recorded movements made by each fish around the experimental arena. The tank was illuminated by two large infrared lamps, situated one half way down each side of the

experimental arena opposite each other. The CCTV camera was Panasonic WV-BP100/B, fitted with a monochrome 1/3 TV lens (Panasonic WV-LA4R5C3A, 45mm 1:1.2) which was infrared sensitive, it could provide us with relatively clear monochrome pictures of the fish against the light background.

Experimental procedure

Fish were introduced to the experimental tank and over a period of several weeks (depending on each fish) were trained to feed in pots for both frozen and live chironomid larvae (bloodworm), only having access to the feeding area at night. The pots were checked each morning, and any remaining food or prey was recorded. When very little food remained on several successive nights, it was clear that individuals had adjusted to feeding from the pots, and I was able to begin the experiments.

The experimental protocol consisted of starving each fish for two days prior to every experimental run, in order to make sure that they were hungry. The evening before each experimental run, all the experimental pots and the coral sand within them were thoroughly washed to remove traces of any previous cues. Into four of the nine pots, ten live bloodworm were added and left for up to one hour. The pots were then placed into the experimental tank, leaving them overnight to settle. A record was kept of the position of each pot containing prey and prior to each experimental run the number of unburied and buried bloodworm in each pot was noted. It was rare to find any completely unburied individuals, but quite common for there to be one or two half buried bloodworm. Directly before each experimental run all the equipment (lights,

camera and video recorder) were checked. Once the aquarium lights were off and the infrared lamps were switched on the partition was lifted and video recording began.

I wanted to screen foraging behaviour when fish were actively foraging, but before foraging activity decreased due to satiation. Videotapes of fish foraging for up to 8 hours were observed, and a sampling period of 2 hours was identified as suitable. At the end of each experimental run, the fish was returned to its living area and the partition replaced. Each pot containing prey was examined for bloodworm and a record was kept of the numbers of bloodworm remaining both buried and unburied.

In total, data were collected on four fish. The following table shows how many repetitions for each fish have been used in subsequent analysis.

Fish number	**Number of repetitions**
1	1
2	1
3	2
4	3
Total 4	7

Video Analysis

Each experimental run provided us with a record of the behaviour of each fish when inside the experimental arena. General observations were made on the behaviour of the fish in addition the following aspects of behaviour were studied:

- The sequence in which each fish visited the pots, and at what time.
- The time spent in close proximity to each pot.
- The time spent foraging within each pot.
- The time spent foraging around the outside of each pot.

The aim was to ascertain whether fish visited some pots more frequently and for longer than others, and if so whether this related to the presence or absence of concealed prey.

Data analysis

We were unable to combine data between the trials, because foraging patterns differed between fish and trials. Instead all the trials have been examined separately, compared and general trends identified. All the data on number of pot visits and visit duration have been taken for the first two hours of each trial. The data on proportion of prey eaten and total foraging durations in pots are taken from the total trial period, which differs in some cases. The data were analysed using a series of both parametric and non-parametric statistics, using a combination of Excel version Microsoft Office '97 and Minitab version 11.

4.3 Results

The foraging behaviour of ruffe in the dark

Ruffe foraging under low light levels were active during most of the foraging period; this is in contrast to the low levels of activity observed under well lit conditions, as seen in the experiments conducted in chapter 5. Fish emerged quickly from the living area, and spent most of their time searching for food both in and around the base of pots; the remaining time was mainly spent moving between pots. Occasionally fish would return to the living area of the tank, which was outside the camera's field of view. These absences from the experimental arena were rarely long, and varied from a few seconds up to 2 minutes. Fish were also seen occasionally swimming up to the water surface, when not searching for food.

When foraging at pots a regular sequence of searching events are often seen. Initially fish would swim up to a pot and stop suddenly, orientating themselves to face the pot. The fish would then begin searching the immediate area outside the pot. Although it was unlikely that there was food present in the area around the pot, it is possible that some of the bloodworms had been able to swim out. After this phase in searching, fish would almost always move back slightly from the pot and then elevate themselves and search inside. Following this, sometimes fish would leave the pot straight away and go on to another, but other times they would remain at the pot and possibly go on to forage inside again.

It was easy to distinguish a fish searching for prey from one that was either inactive, or just swimming around, as ruffe displayed distinct behaviours when searching for

prey within the tank substrate and pots. While searching for prey outside the pots ruffe elevate but remain relatively close to the substrate, lifting their tail upwards so that their body is at an angle from the tank base. The body is kept relatively still and the pectoral fins are used to manoeuvre in both a horizontal and vertical plane. The foraging behaviour observed from individuals when foraging in the pots was slightly different from this, probably due to the presence of the pot limiting the movement of the fish. Fish foraging within pots were at a much sharper angle from the substrate (sometimes almost at 90° from it); this allowed them to clear the lip of the pots, in order to forage in the substrate. During foraging visits inside pots ruffe would work around in a circle, starting at one point and foraging, keeping their head in the pot and moving their body around the pot in either a clockwise or anti-clockwise direction, occasionally lunging at the pot base. Fish made two different types of trips to pots. Some involved long and vigorous periods of searching in and around the pot, while others were very short visits, where fish did not forage vigorously, and left quickly.

How efficient are ruffe at foraging for concealed prey in the dark?

In general, the longer fish spent at each pot and the more visits it made, the greater percentage of prey were taken, as would be expected (see figure 4.1 to 4.5). However for some fish, foraging efficiency (the percentage of prey taken in relation to time spent foraging) was very different for different pots. For example, fish 3 in trial 7 (figure 4.1a) spent more time foraging in pot 3C (where it only ate 40 percent of the prey), than in pot 2C where 80 percent of prey were eaten). Fish 4 in trial 11 spent nearly twice as long foraging in pot 1C, where only 30 percent of the prey were taken, than it did in 2B where 60 percent were eaten (figure 4.3a). The same fish foraged

Figure 4.1a

Comparison of total foraging duration with the percentage of prey consumed, fish 3, experiment 7.

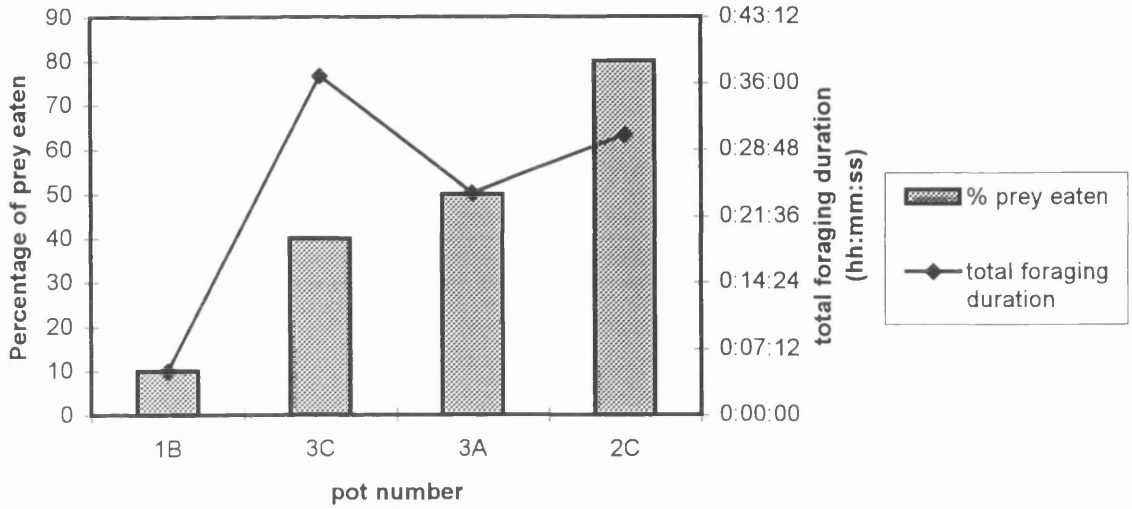


Figure 4.1b

Comparison of the total number of foraging visits with the percentage of prey consumed, fish 3, experiment 7.

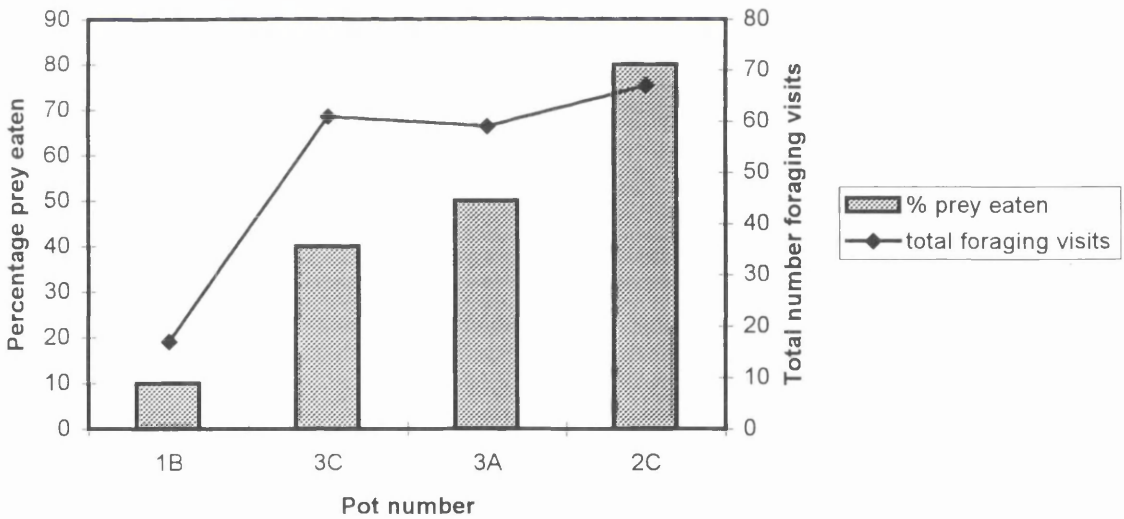


Figure 4.2a

Comparison of total foraging duration with the percentage of prey consumed, fish 3, experiment 9.

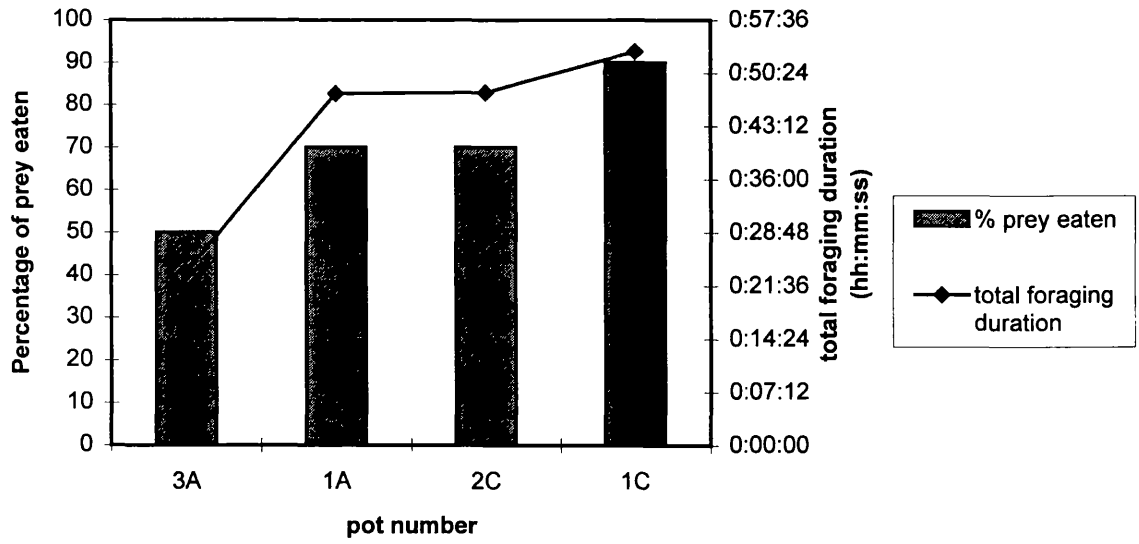


Figure 4.2b

Comparison of the total number of foraging visits with the percentage of prey consumed, fish 3, experiment 9.

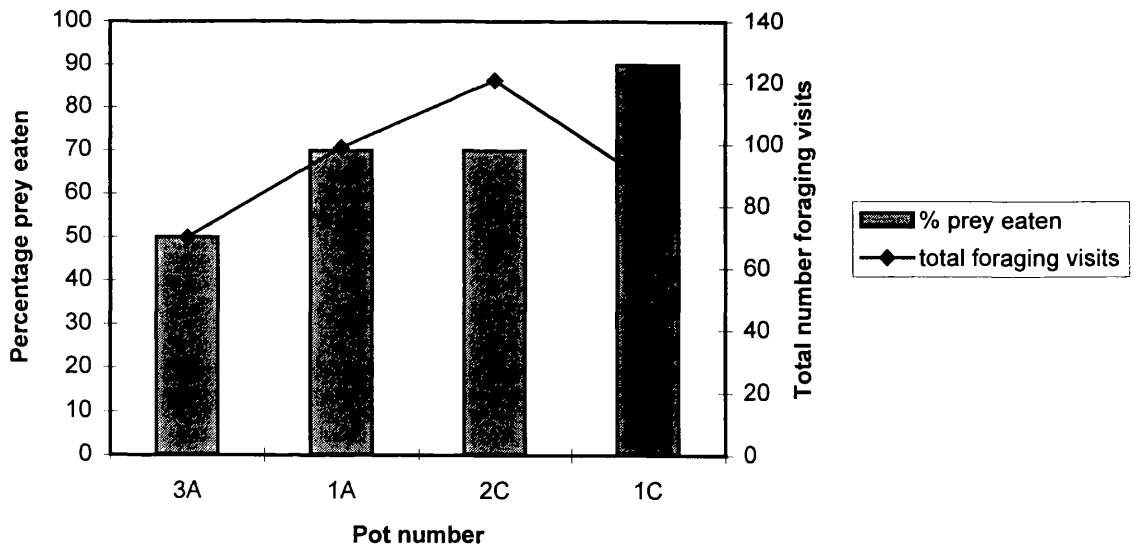


Figure 4.3a

Comparison of total foraging duration with the percentage of prey consumed, fish 4, experiment 11.

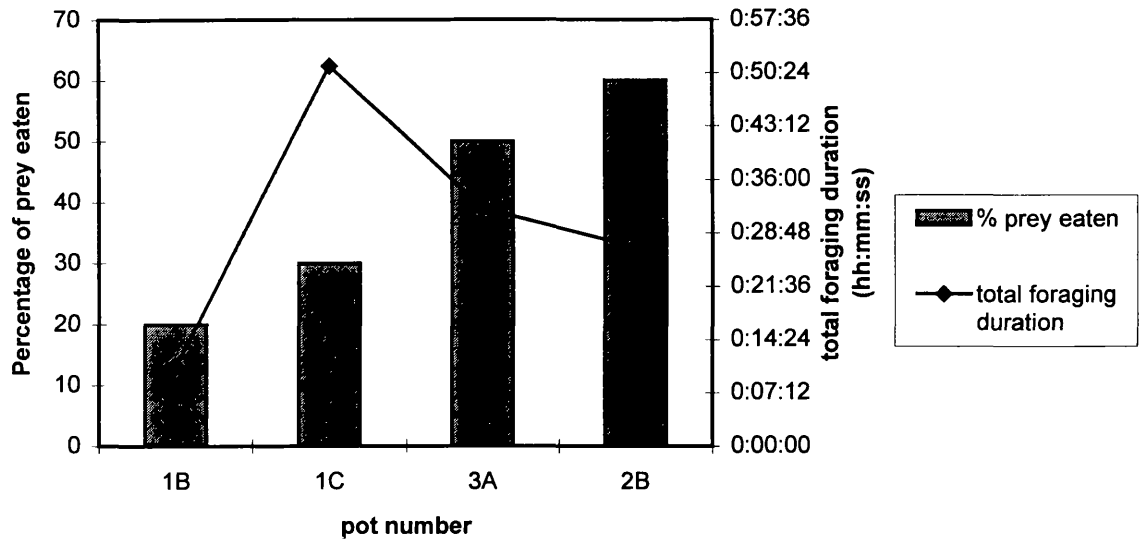


Figure 4.3b

Comparison of the total number of foraging visits with the percentage of prey consumed, fish 4, experiment 11.

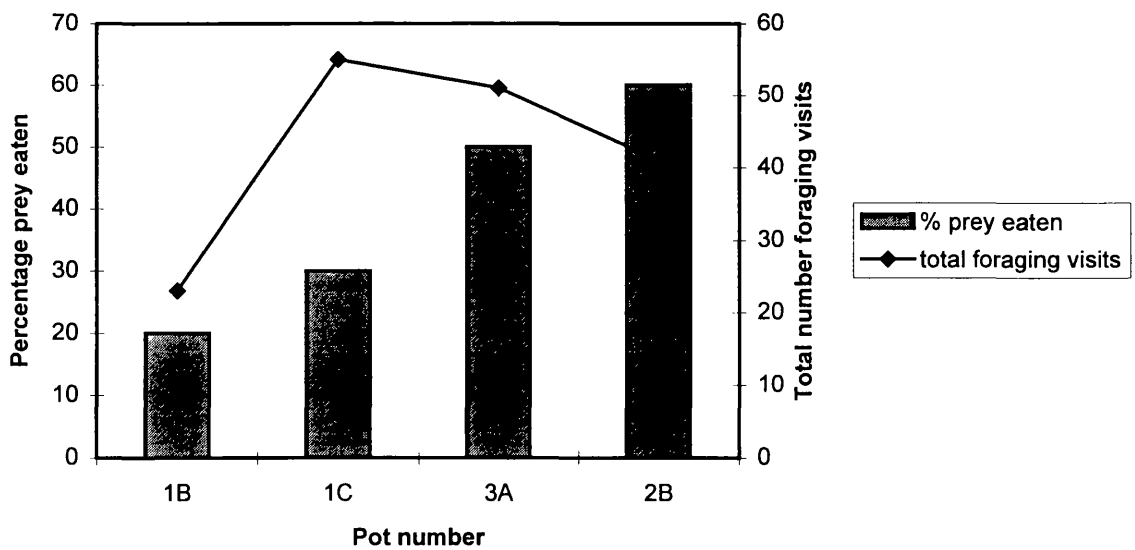


Figure 4.4a

Comparison of total foraging duration with the percentage of prey consumed, fish 4, experiment 12.

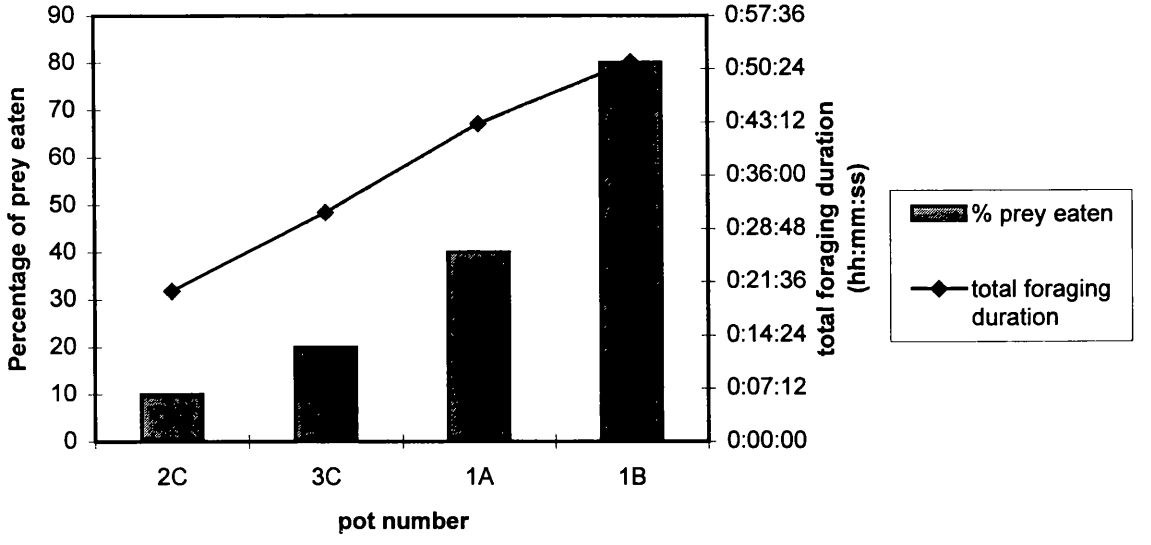


Figure 4.4b

Comparison of the total number of foraging visits with the percentage of prey consumed, fish 4, experiment 12.

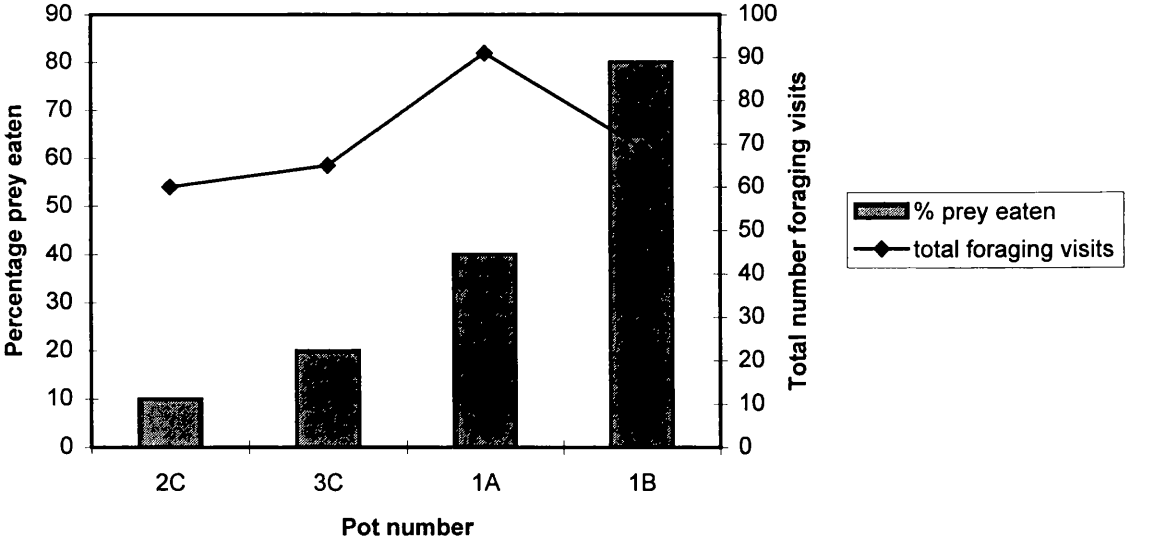


Figure 4.5a

Comparison of total foraging duration with the percentage of prey consumed, fish 4, experiment 13.

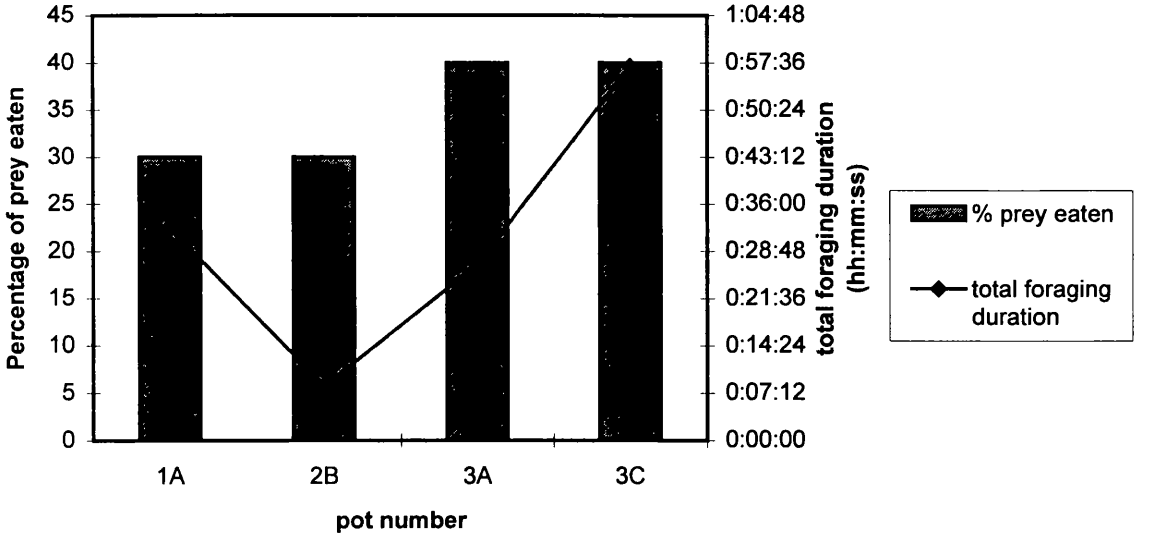
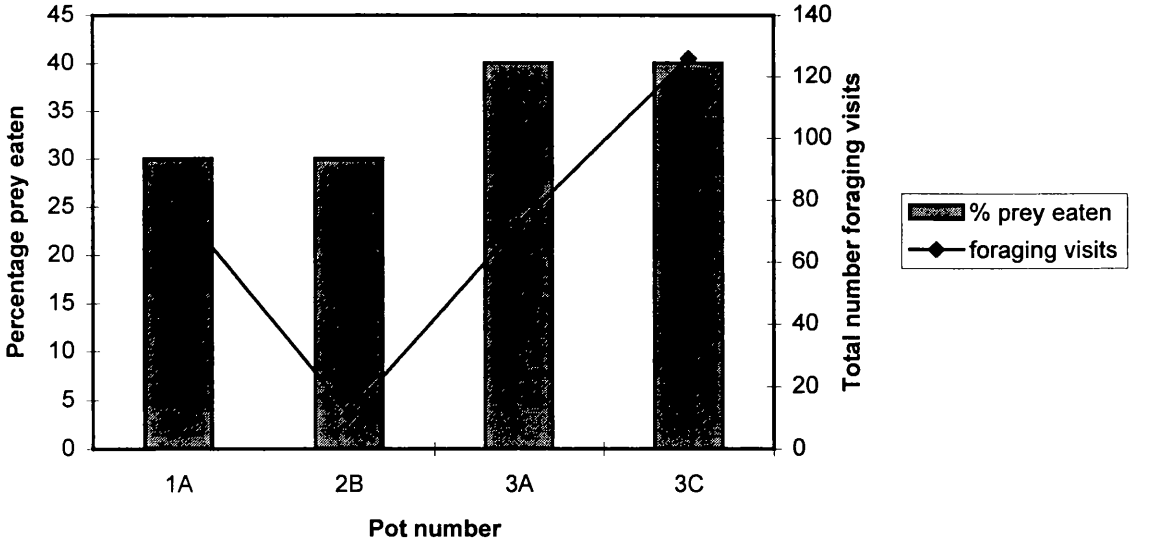


Figure 4.5b

Comparison of the total number of foraging visits with the percentage of prey consumed, fish 4, experiment 13.



very inefficiently in trial 12 (figure 4.4a), but in trial 13 showed a very large variation in foraging efficiency, spending under 10 minutes in pot 2B and obtaining 30 percent of the available prey, whilst spending up to one hour foraging for only 40 percent of the bloodworm from pot 3C (figure 4.5a). Since the allocation of prey to pots was semi-random, there are not sufficient data to examine the causes of this variability, although the pot in the centre of the foraging arena (pot 2B) was foraged very efficiently on two occasions by fish 4.

Frequency of visits

To examine the relative frequency of foraging visits to pots with and without food, Chi-squared tests were carried out on the total number of visits to each category of pot in each session. In sessions fish 1, trial 2 (Table 4.6); trial 7 (Table 4.8), trial 9 (Table 4.9) and trial 13 (Table 4.12), the overall distribution was different from random, pots with food being visited significantly more frequently. For the remaining sessions, pots with food were visited more often but this was not significant. In addition, cumulative visit frequencies were plotted against time (in 15 minute blocks). These tests and data are shown in tables 4.6 to 4.12 and figures 4.6 to 4.12, respectively.

In all cases the cumulative foraging visits rose gradually, indicating a steady foraging effort through the trial. However for a subset of fish, those for which the overall distribution of visits to pots with and without food were significantly different, the slope is greater for pots with food (shown in Figures 4.6 to 4.12). This indicates that preferences for pots with food increases with time and that for some fish learning may occur throughout the experimental period.

Figure 4.6a

Comparison of the observed and expected number of cumulative foraging visits to pots containing food, fish 1, experiment 2.

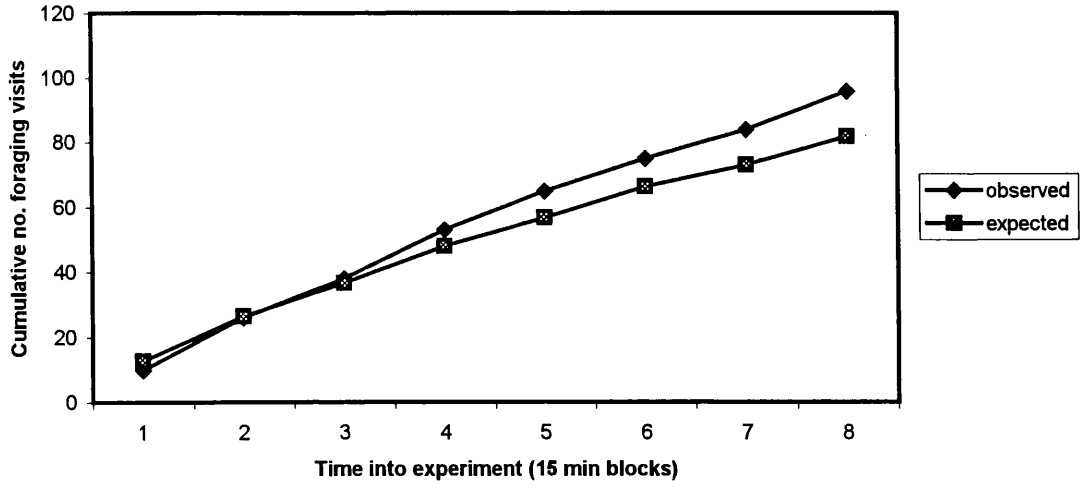


Figure 4.6b

Comparison of the observed and expected number of cumulative foraging visits to pots containing no food, fish 1, experiment 2.

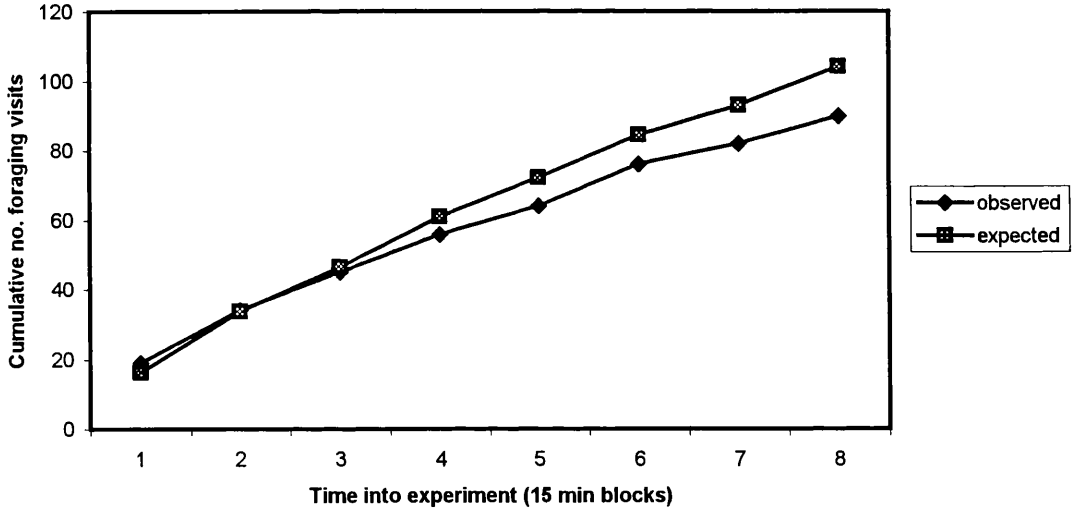


Table 4.6

Total number of foraging visits to pots with and without food

with food	without food	d.f	χ^2	P
96	90	1	4.27	<0.05

Figure 4.7a

Comparison of the observed and expected number of cumulative foraging visits to pots containing food, fish 2, experiment 2.

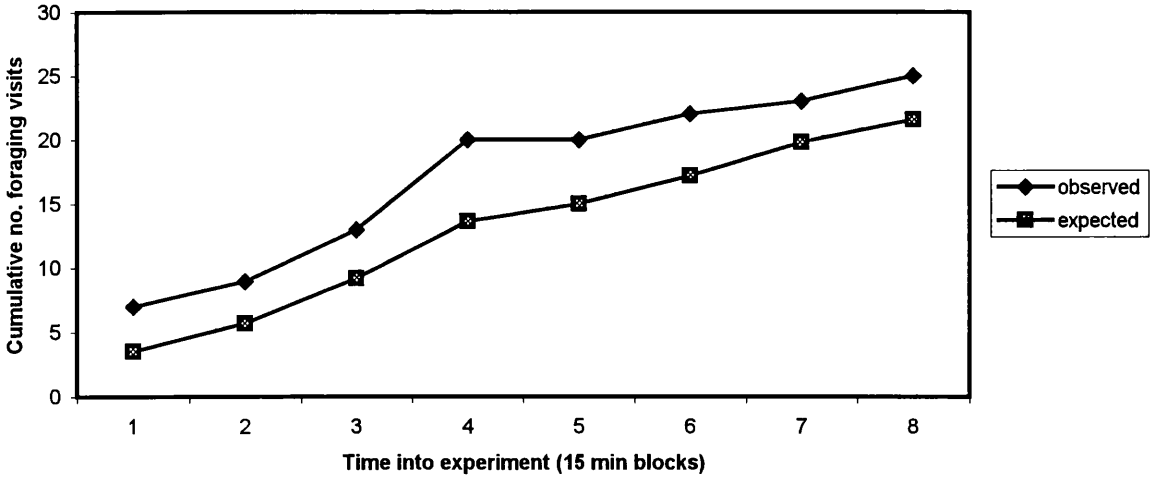


Figure 4.7b

Comparison of the observed and expected number of cumulative foraging visits to pots containing no food, fish 2, experiment 2.

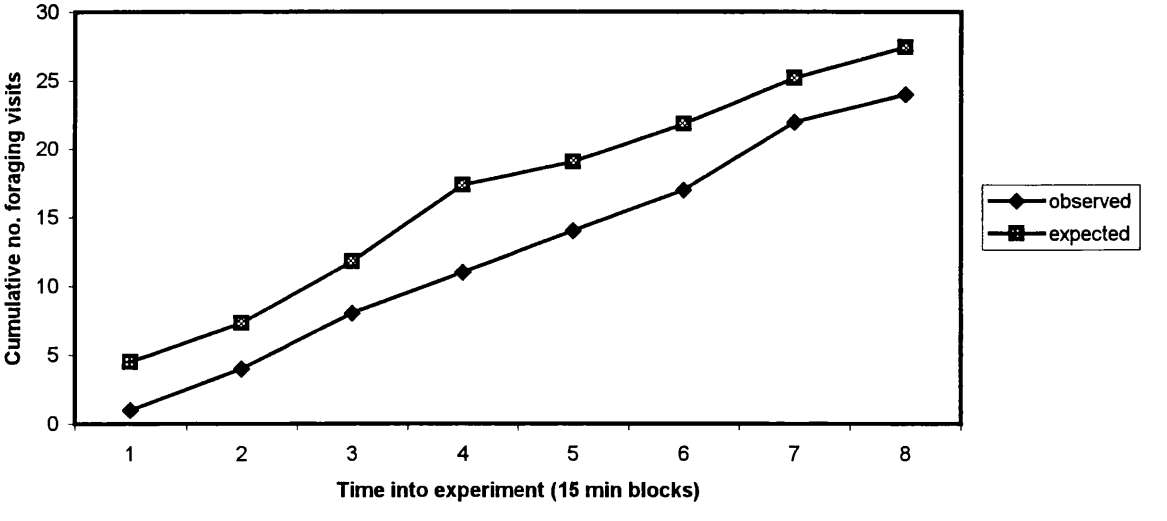


Table 4.7

Total number of foraging visits to pots with and without food

with food	without food	d.f	$\chi 2$	P
25	24	1	0.74	<0.50

Figure 4.8a

Comparison of the observed and expected number of cumulative foraging visits to pots containing food, fish 3, experiment 7.

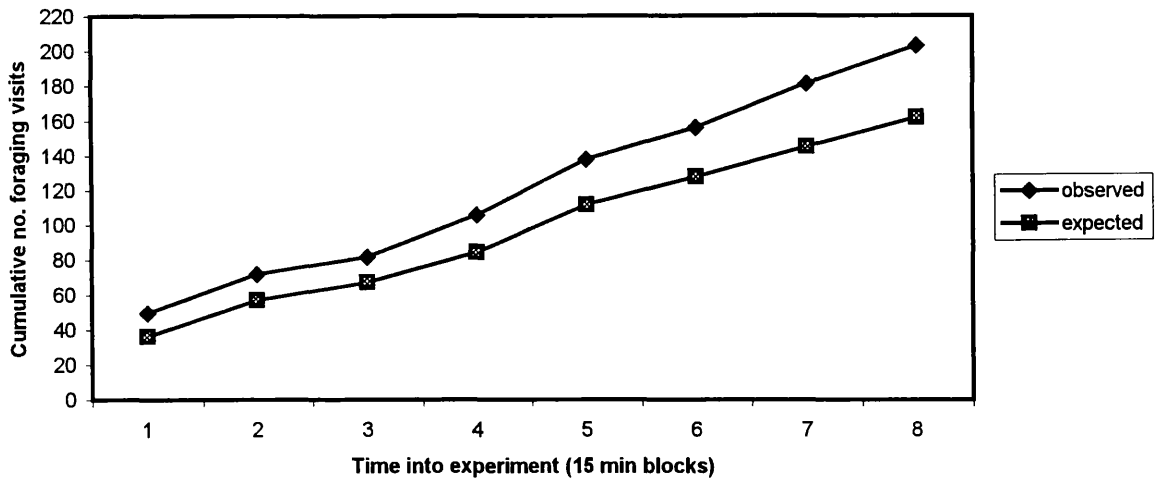


Figure 4.8b

Comparison of the observed and expected number of cumulative foraging visits to pots containing no food, fish 3, experiment 7.

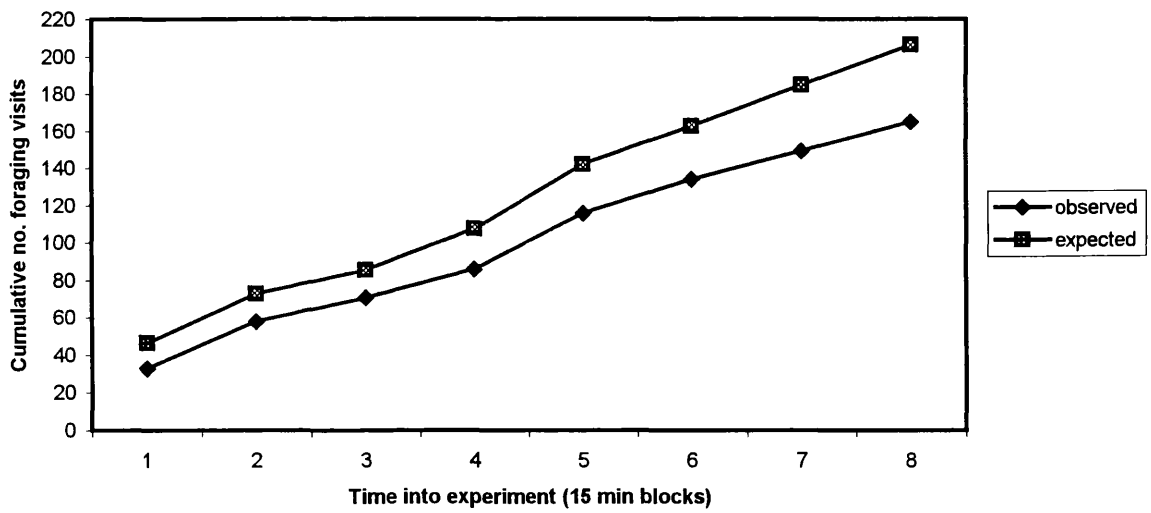


Table 4.8

Total number of foraging visits to pots with and without food

with food	without food	d.f	$\chi 2$	P
203	165	1	18.54	<0.001

Figure 4.9a

Comparison of the observed and expected number of cumulative foraging visits to pots containing food, fish 3, experiment 9.

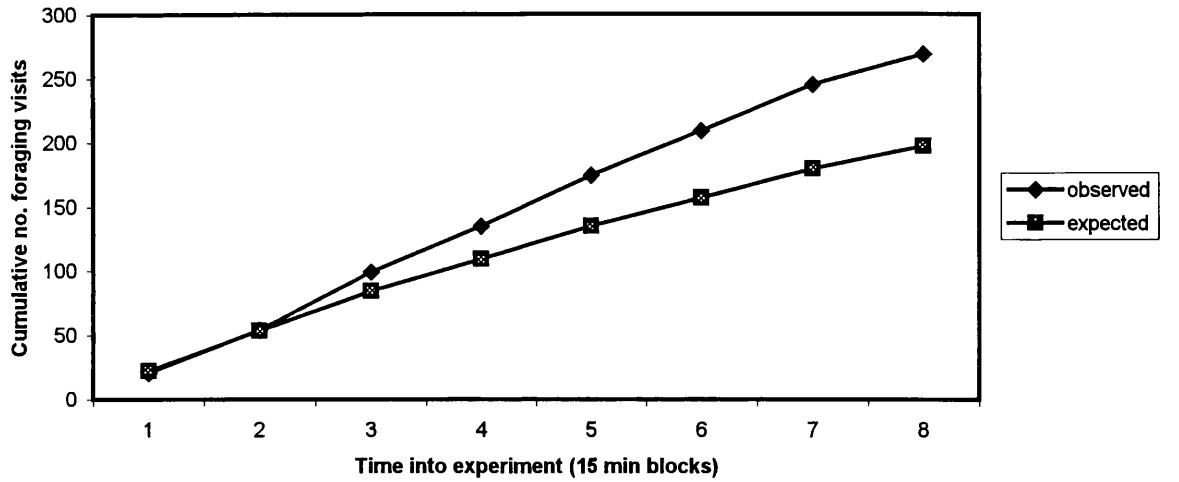


Figure 4.9b

Comparison of the observed and expected number of cumulative foraging visits to pots containing no food, fish 3, experiment 9.

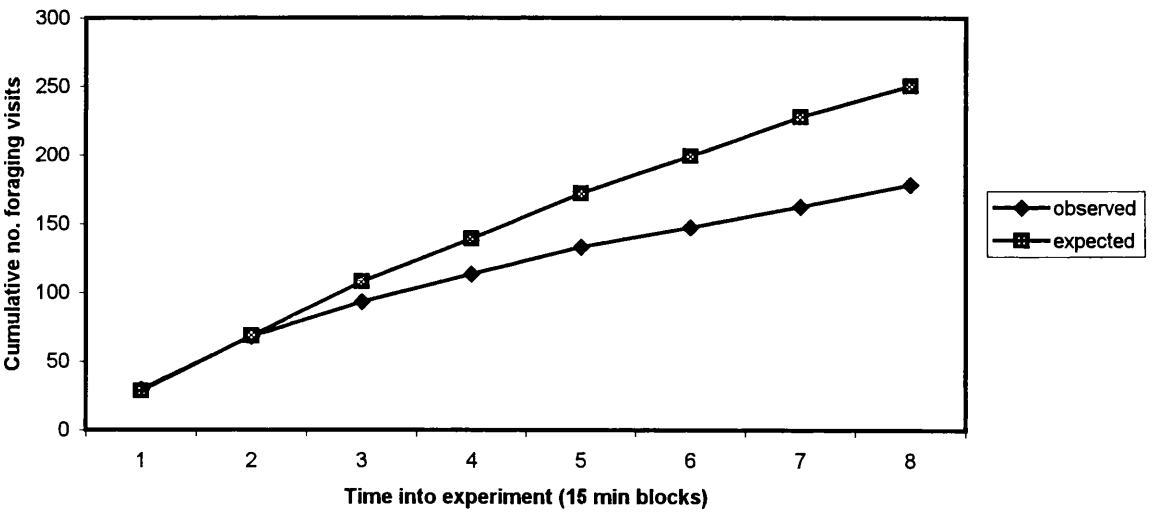


Table 4.9

Total number of foraging visits to pots with and without food

with food	without food	d.f	χ^2	P
269	178	1	47.05	<0.001

Figure 4.10a

Comparison of the observed and expected number of cumulative foraging visits to pots containing food, fish 4, experiment 11.

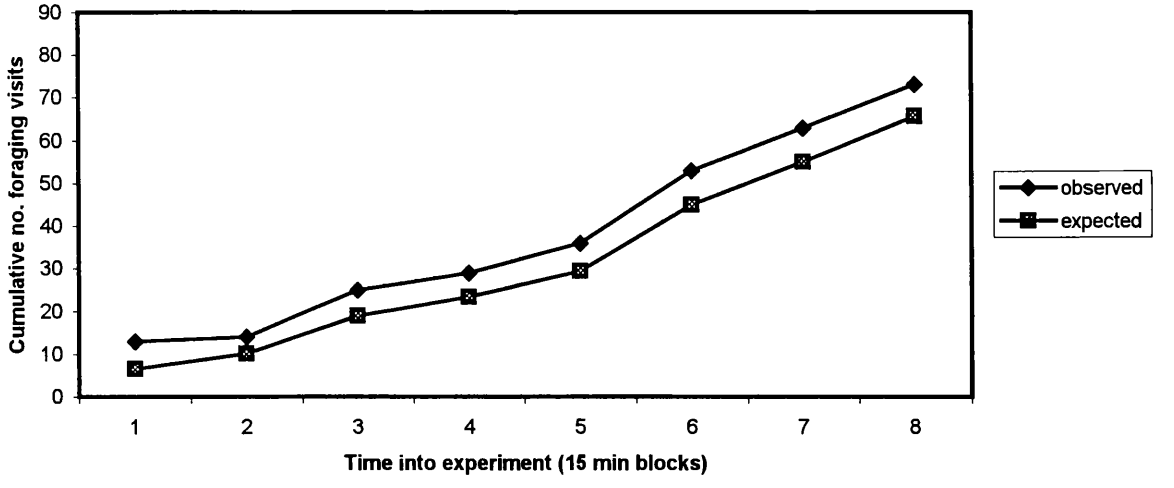


Figure 4.10b

Comparison of the observed and expected number of cumulative foraging visits to pots containing no food, fish 4, experiment 11.

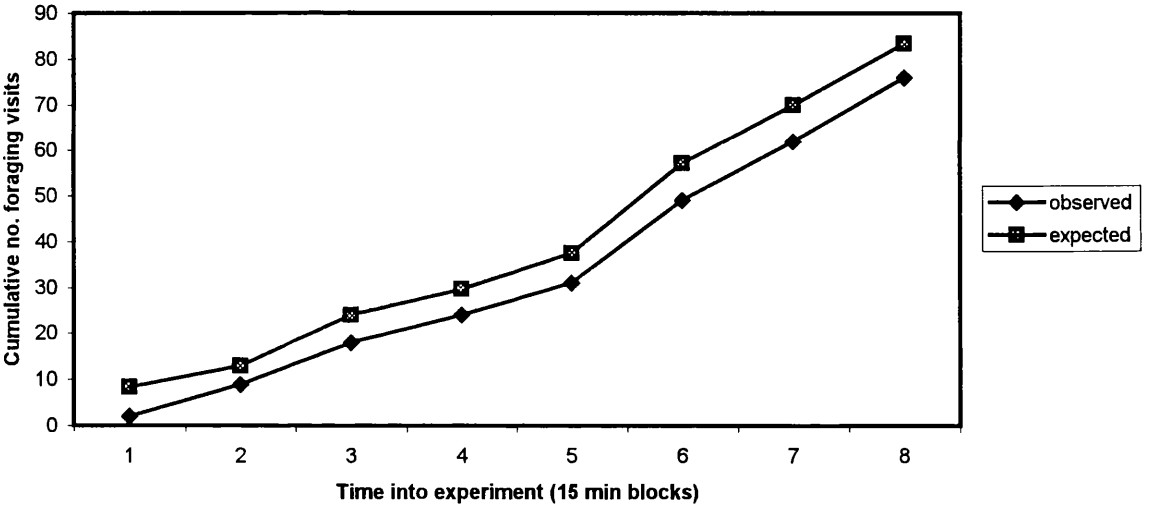


Table 4.10

Total number of foraging visits to pots with and without food

with food	without food	d.f	$\chi 2$	P
73	76	1	1.33	<0.25

Figure 4.11a

Comparison of the observed and expected number of cumulative foraging visits to pots containing food, fish 4, experiment 12.

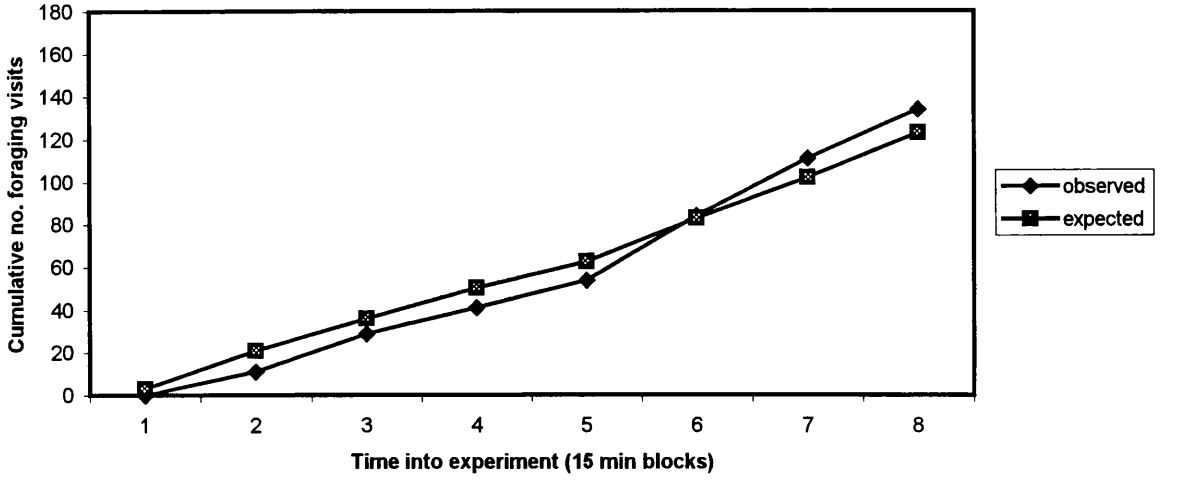


Figure 4.11b

Comparison of the observed and expected number of cumulative foraging visits to pots containing no food, fish 4, experiment 12.

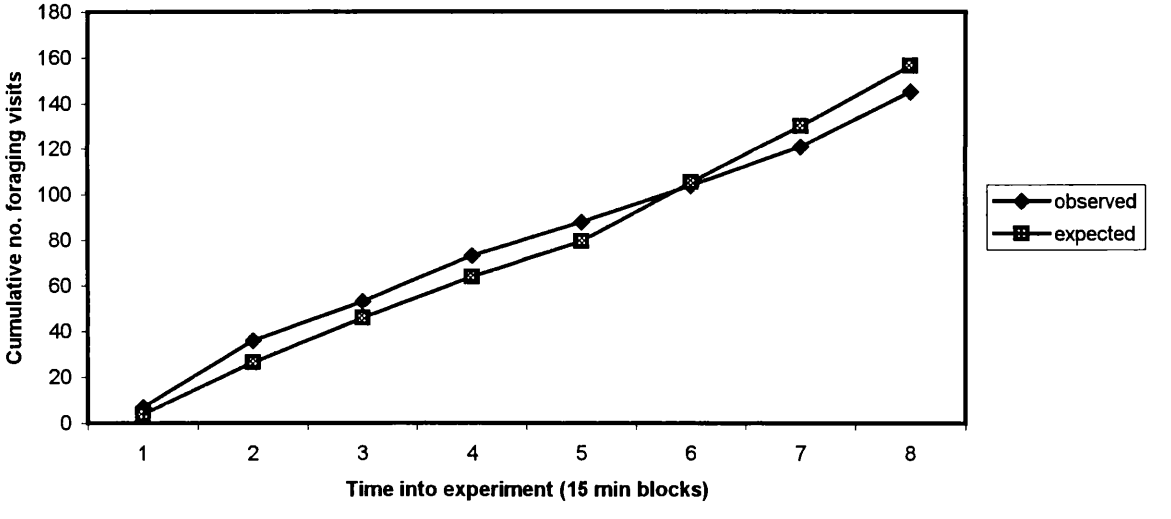


Table 4.11

Total number of foraging visits to pots with and without food

with food	without food	d.f	$\chi 2$	P
134	145	1	1.76	<0.25

Figure 4.12a

Comparison of the observed and expected number of cumulative foraging visits to pots containing food, fish 4, experiment 13.

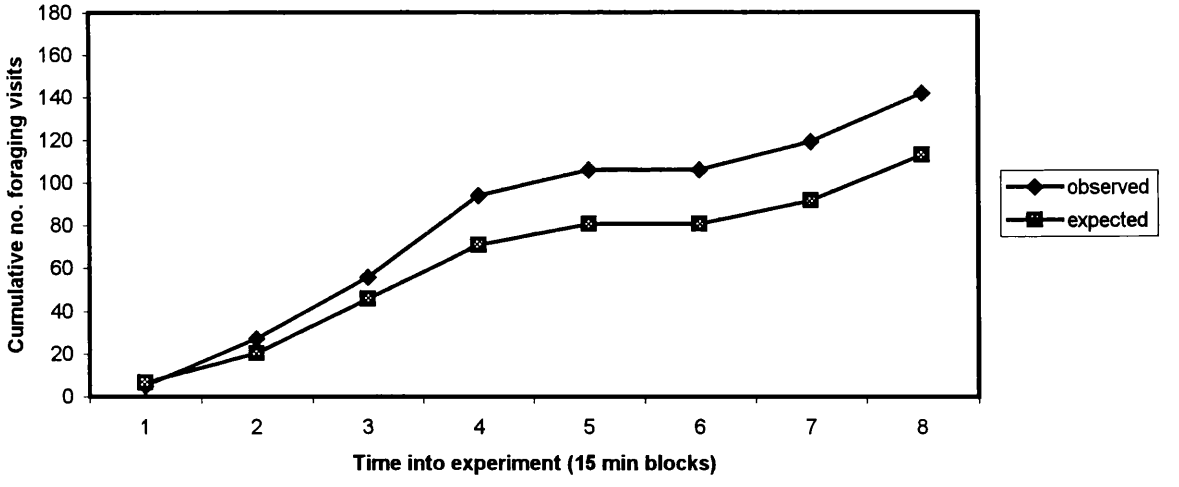


Figure 4.12b

Comparison of the observed and expected number of cumulative foraging visits to pots containing no food, fish 4, experiment 13.

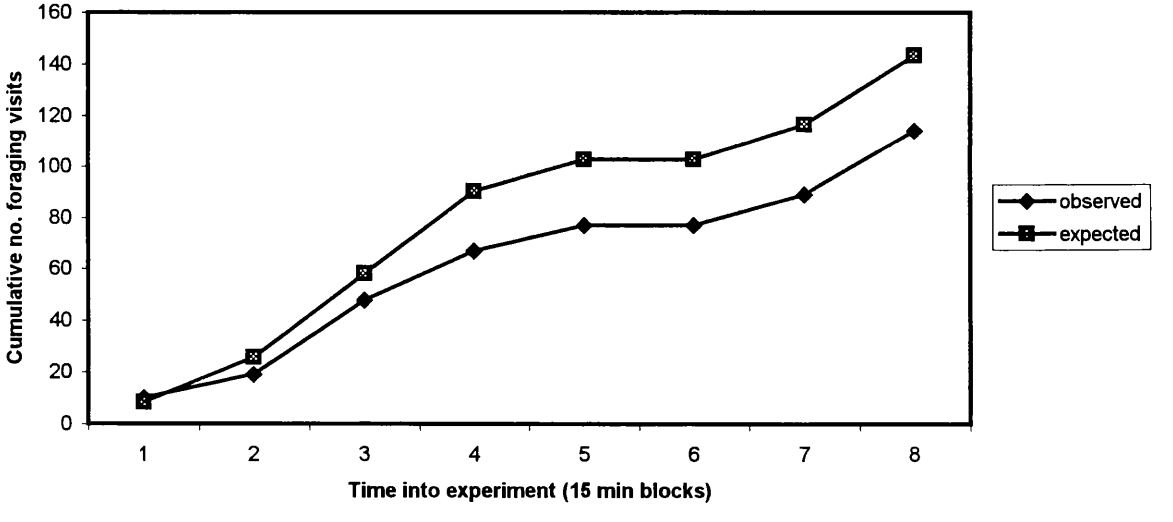


Table 4.13

Total number of foraging visits to pots with and without food

with food	without food	d.f	$\chi 2$	P
142	114	1	13.32	<0.001

The mean duration of pot visits (in seconds) was plotted against time, for trials which had a significant Chi-squared result (fish 1, trial 2 and trials 7,9 and 13) (Figure 4.20) and those which did not (fish 2, trial 2, and trials 11 and 12) (Figure 4.21). Individuals that did not visit significantly more food pots differed from those that did show significant differences, with respect to visit duration, specifically they spent longer at pots with food in than did the other foraging category.

Duration of visits to pots with and without food

Overall the ruffe in this study visited pots with food consistently more often than pots without food. T-tests showed that inside pot foraging visits lasted significantly longer in pots with prey in than those with no food source (see table 4.13 for results of analyses). There was no difference in the duration of outside foraging visits made to pots with and without food. It is possible that visits where fish appear to be foraging around the outside of pots actually involved individuals investigating whether any food cues are present, before attempting to forage inside a pot.

Temporal trends in duration of foraging visits inside and outside the pots are shown in figures 4.13 to 4.19. The results of the analysis by ANOVA and *a posteriori* tests are shown in Table 4.14. For pots without food in most sessions, visit duration (for both inside and outside pots) does not change markedly with time. The exceptions are fish 1, trial 2 (Figure 4.13a), indicates there is a slight incremental trend, fish 3, trial 7 (Figure 4.15a), in which durations were erratically high in some pots and fish 4, trial 13 (Figure 4.19a), in which duration decreased with time. For visits to pots with food, in most cases the duration of foraging visits changes significantly with time. The

general trend was for durations to be longer towards the end of the trial, although this was erratic.

Table 4.13

T-test results on duration spent foraging in pots with and without food

Fish / Trial	Visit type	Mean ±S.E, with food	Mean ±S.E, without food	d.f	T	P
1 / 2	Inside	27.5 ± 2.1	15.27 ± 0.96	133	5.36	<0.0001
	Outside	N/A	N/A	N/A	N/A	N/A
2 / 2	Inside	49.4 ± 7.9	21.3 ± 3.0	30	3.32	=0.002
	Outside	N/A	N/A	N/A	N/A	N/A
3 / 7	Inside	28.5 ± 3.0	13.21 ± 1.2	113	4.73	<0.0001
	Outside	19.7 ± 4.9	16.7 ± 4.2	31	0.46	=0.65
3 / 9	Inside	21.8 ± 1.4	16.2 ± 1.3	191	2.91	=0.004
	Outside	23.5 ± 1.4	26.8 ± 9.8	100	-0.33	=0.74
4 / 11	Inside	62.3 ± 12.0	22.7 ± 3.9	16	3.03	=0.008
	Outside	25.6 ± 6.5	14.9 ± 2.4	39	1.56	=0.13
4 / 12	Inside	33.3 ± 3.0	23.0 ± 2.2	79	2.82	=0.006
	Outside	9.38 ± 1.2	8.05 ± 1.4	72	0.73	=0.47
4 / 13	Inside	29.8 ± 3.3	21.4 ± 2.2	82	2.14	=0.036
	Outside	9.39 ± 1.3	8.46 ± 1.4	30	0.48	=0.64

Table 4.14

Intensity of foraging effort at pots with and without food.

Results from ANOVA on duration of foraging visits.

					Significant differences from Tukey's pariwise comparisons	
Fish no.	Trial no.	Type of visit	Pots with food	Pots without food	Pots with food	Pots without food
1	2	Inside	$F_{7,88}=5.71$ $P<0.001$	$F_{7,82}=2.67$ $P=0.015$	1/5, 1/7, 2/7, 4/7, 6/7, 7/8	1/6
		outside	N/A	N/A		
2	2	Inside	$F_{6,18}=13.6$ $P<0.001$	$F_{7,16}=0.78$ $P=0.61$	1/3, 1/6, 1/7, 2/6, 2/7, 3/7, 4/6, 4/7, 6/8, 7/8,	
		outside	N/A	N/A		
3	7	Inside	$F_{7,79}=5.88$ $P<0.001$	$F_{7,58}=1.58$ $P=0.16$	1/4, 1/6, 1/7, 2/4, 4/5	
		outside	$F_{7,18}=2.01$ $P=0.11$	$F_{3,7}=27.18$ $P<0.001$		2/4, 2/8, 3/4, 3/8
3	9	Inside	$F_{7,118}=2.69$ $P=0.01$	$F_{7,67}=1.77$ $P=0.11$	2/8, 3/8	
		outside	$F_{7,117}=1.57$ $P=0.15$	$F_{7,90}=0.46$ $P=0.86$		
4	11	Inside	$F_{5,9}=5.29$ $P=0.015$	$F_{5,9}=0.26$ $P=0.93$	5/6, 5/7, 5/8	
		outside	$F_{7,24}=0.92$ $P=0.51$	$F_{7,18}=1.31$ $P=0.30$		
4	12	Inside	$F_{6,37}=1.47$ $P=0.22$	$F_{7,37}=1.49$ $P=0.20$		
		outside	$F_{6,32}=1.47$ $P=0.22$	$F_{7,30}=1.22$ $P=0.32$		
4	13	Inside	$F_{6,42}=1.73$ $P=0.14$	$F_{6,39}=3.93$ $P=0.004$		1/3, 1/4, 1/7, 1/8
		outside	$F_{6,24}=0.96$ $P=0.47$	$F_{5,7}=0.86$ $P=0.55$		

Figure 4.13a

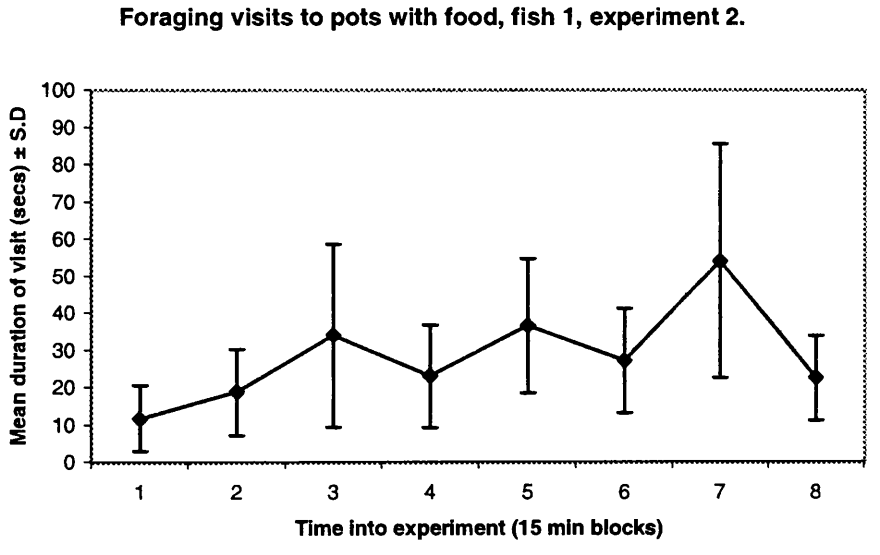


Figure 4.13b

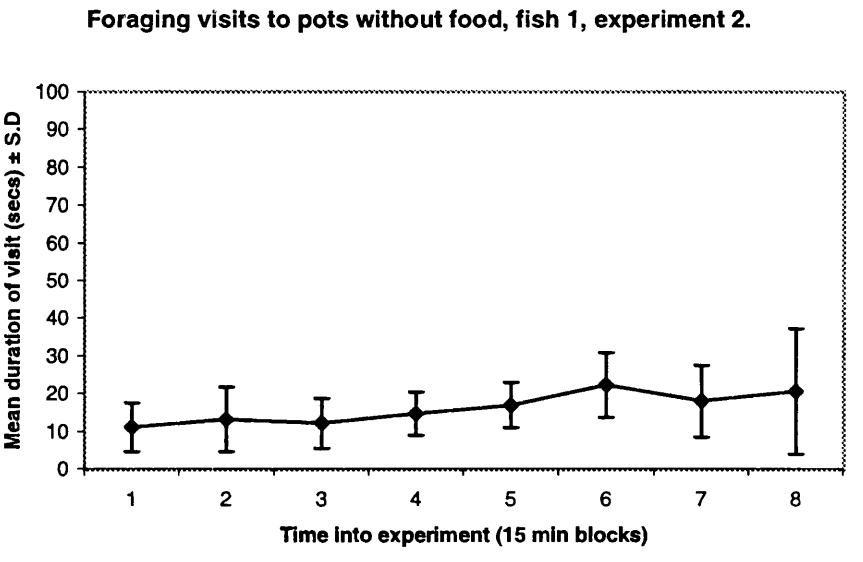


Figure 4.14a

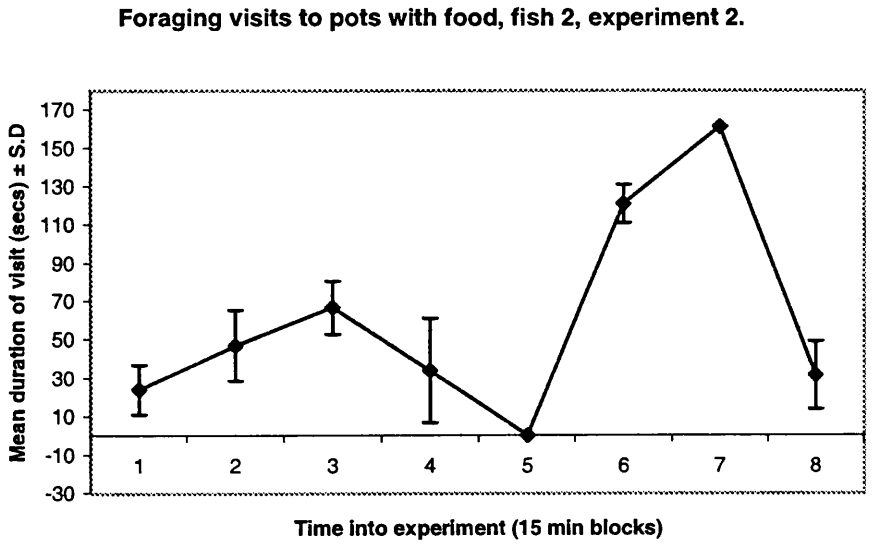


Figure 4.14b

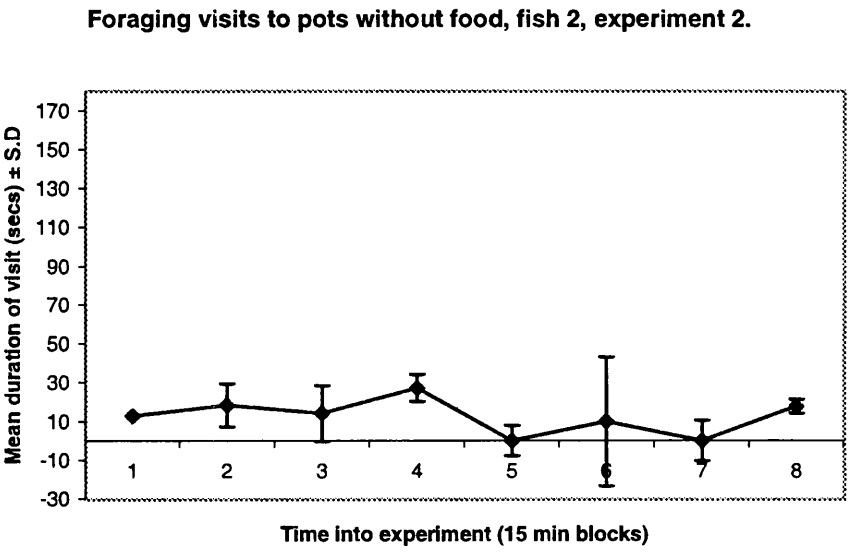


Figure 4.15a

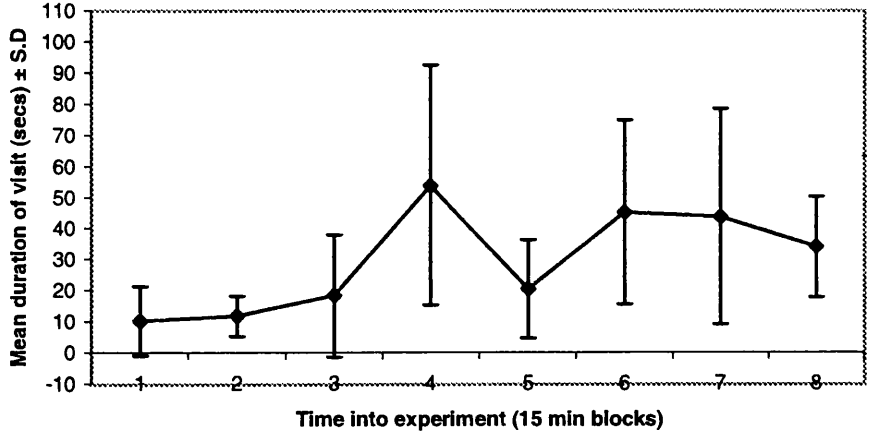


Figure 4.15b

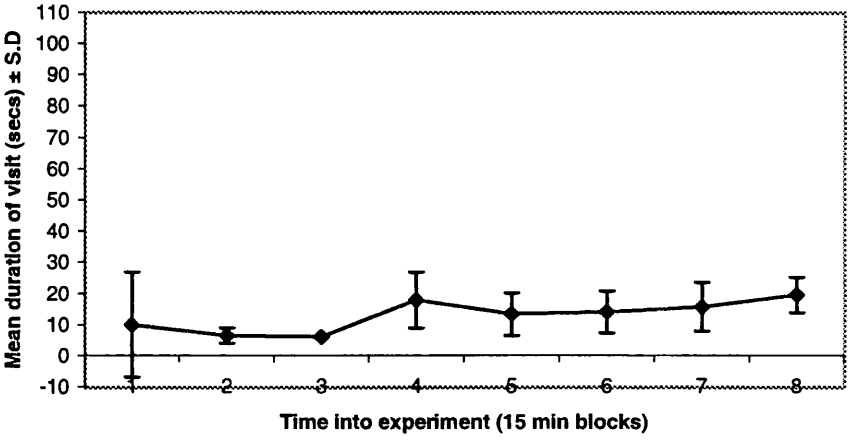


Figure 4.15c

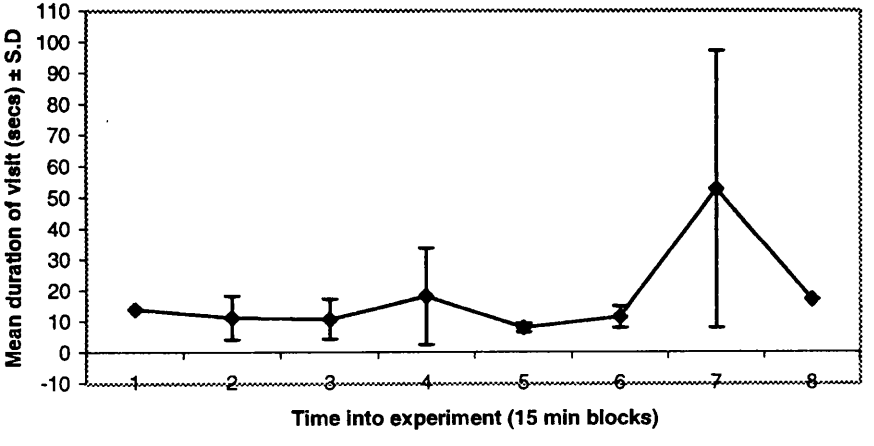


Figure 4.15d

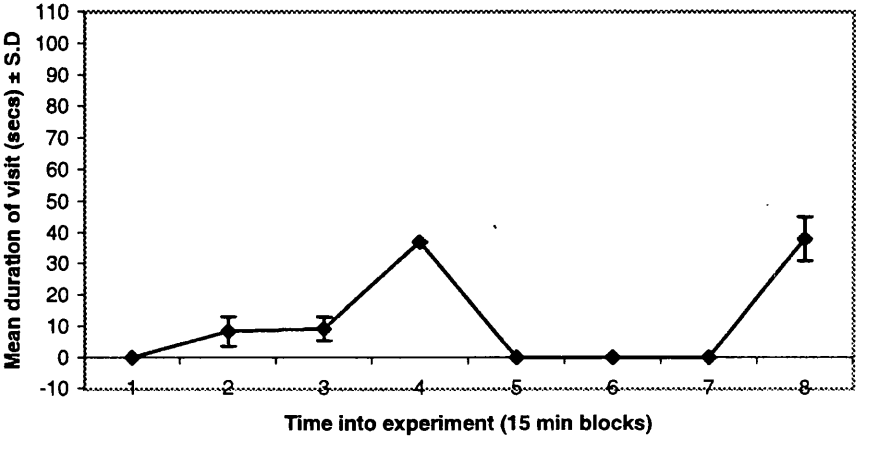


Figure 4.16a

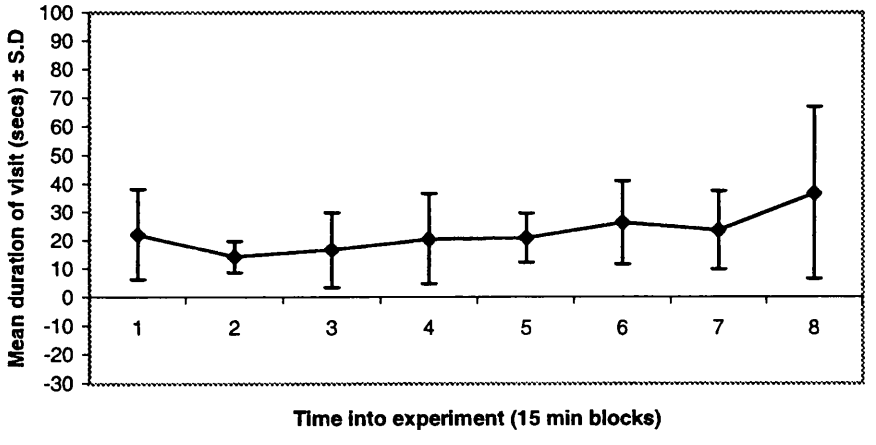


Figure 4.16b

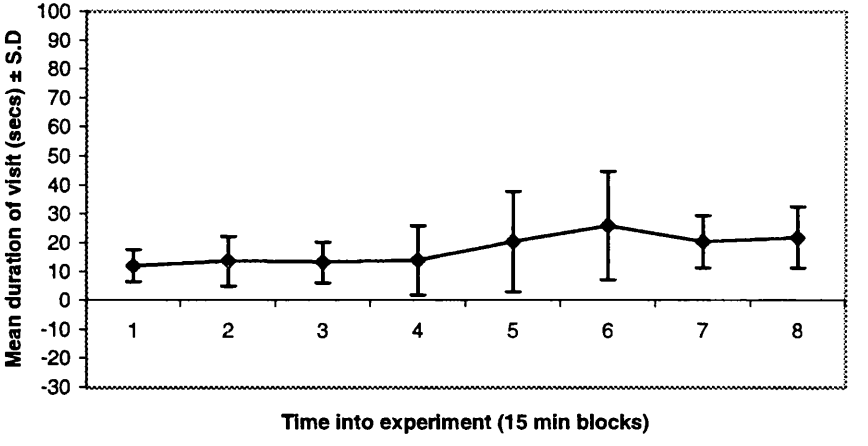


Figure 4.16c

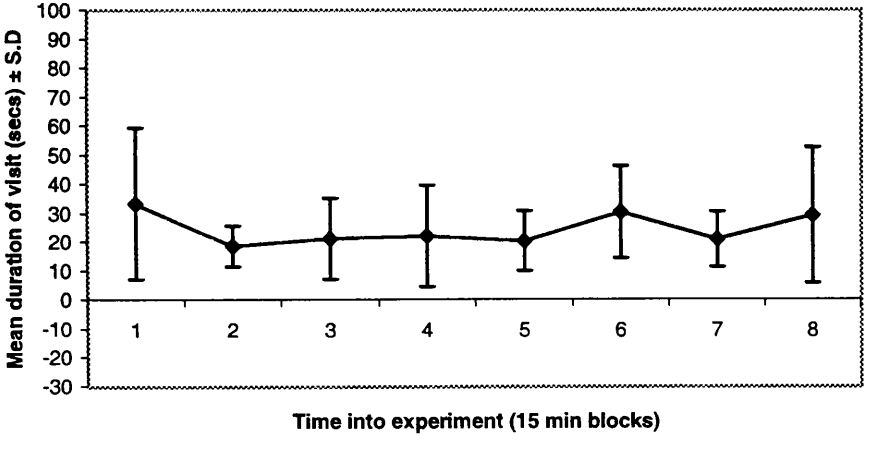


Figure 4.16d

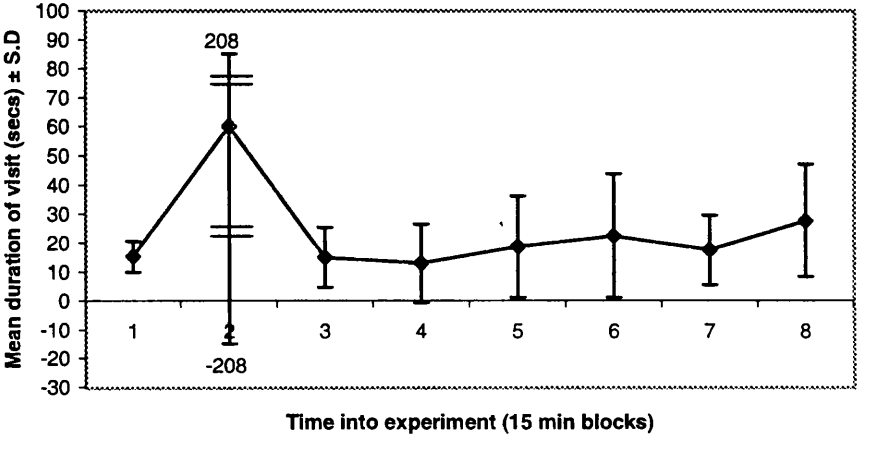
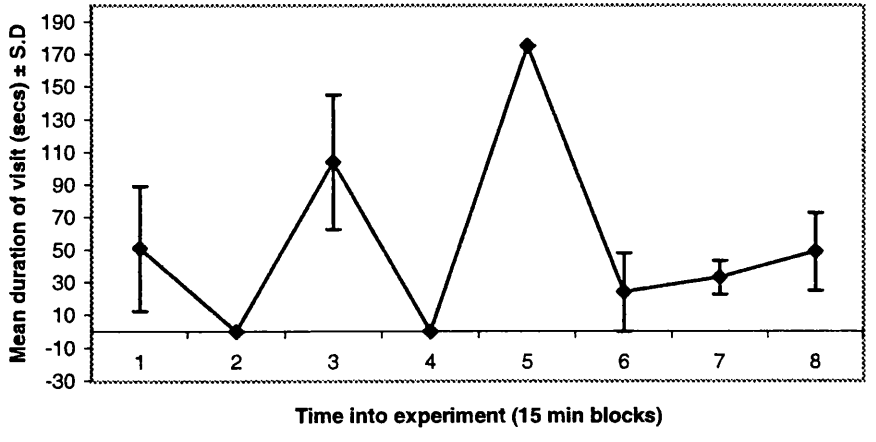


Figure 4.17a



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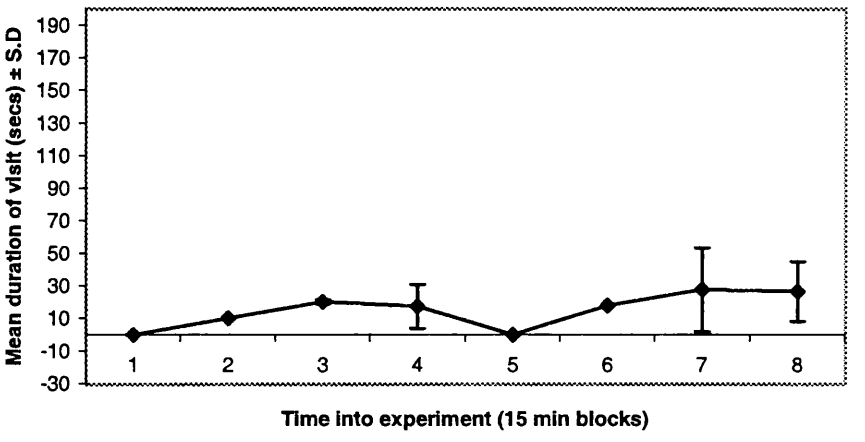

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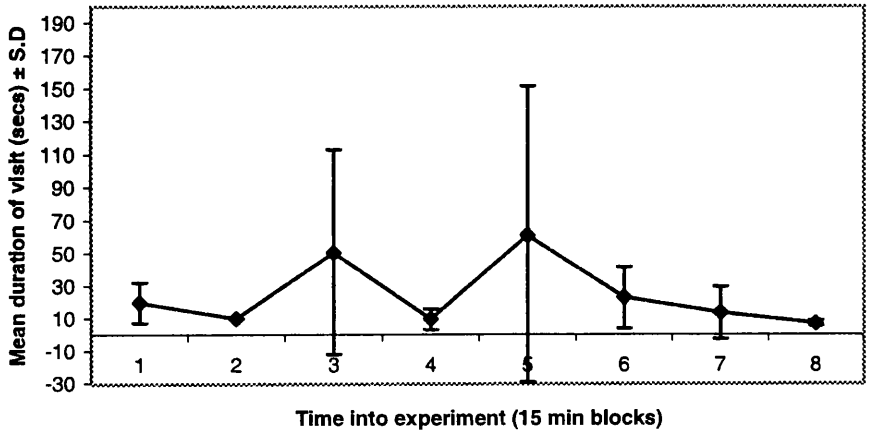
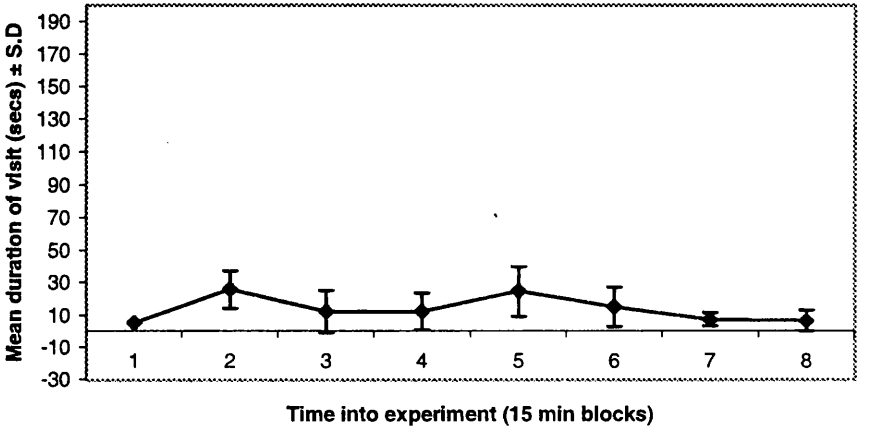


Figure 4.17d



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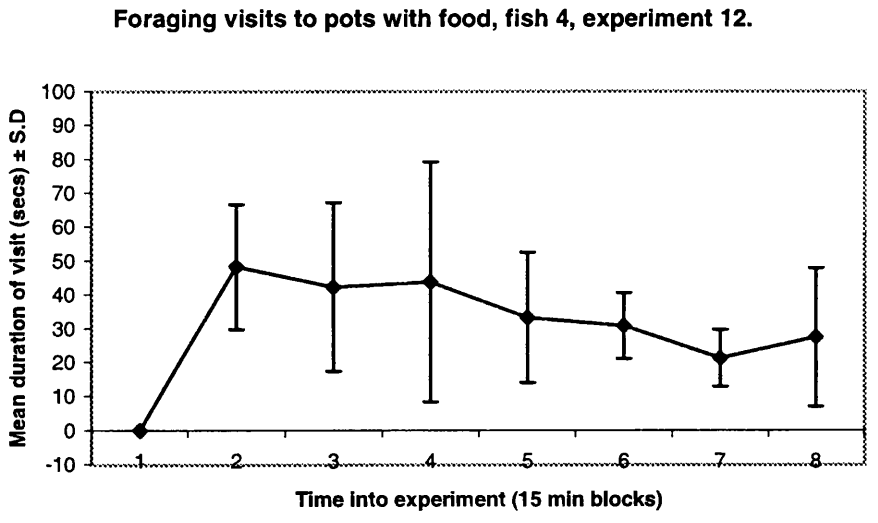
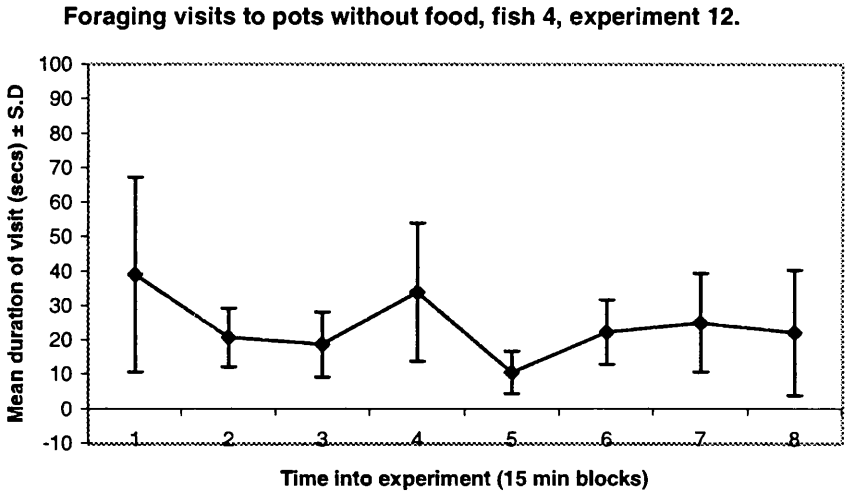


Figure 4.18b



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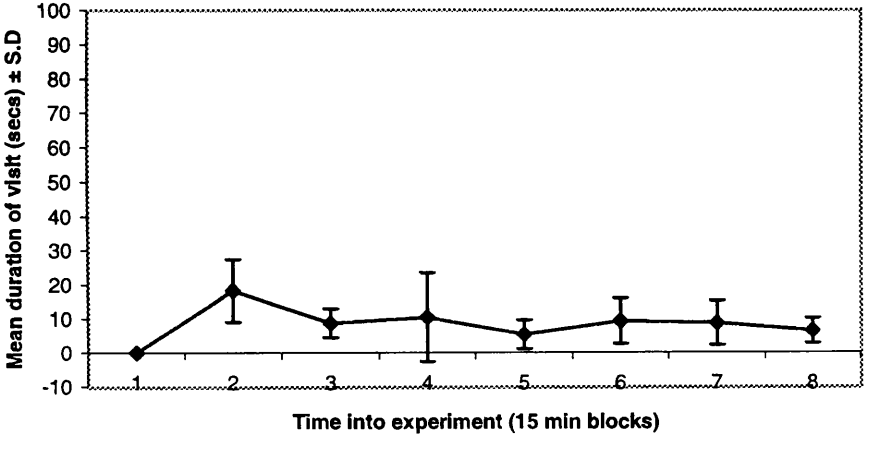

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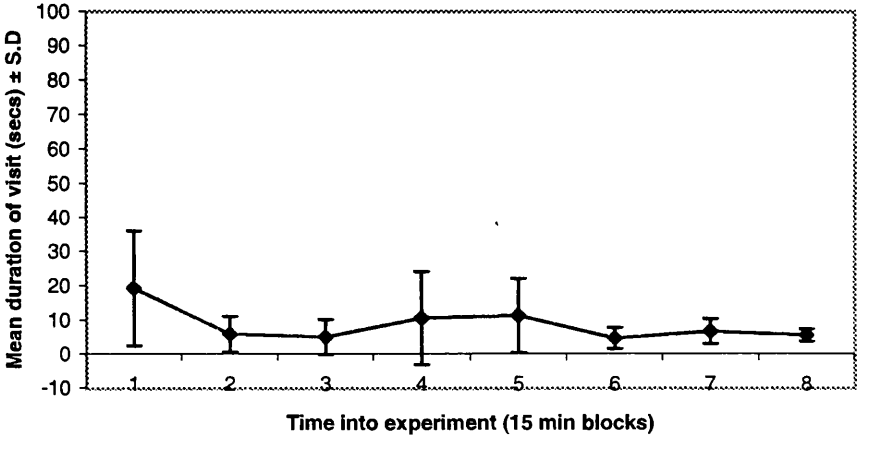


Figure 4.19a

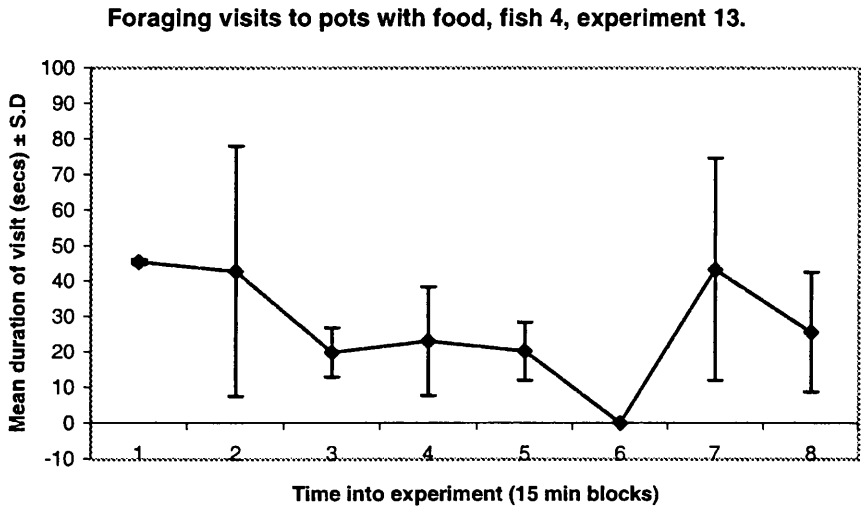


Figure 4.19b

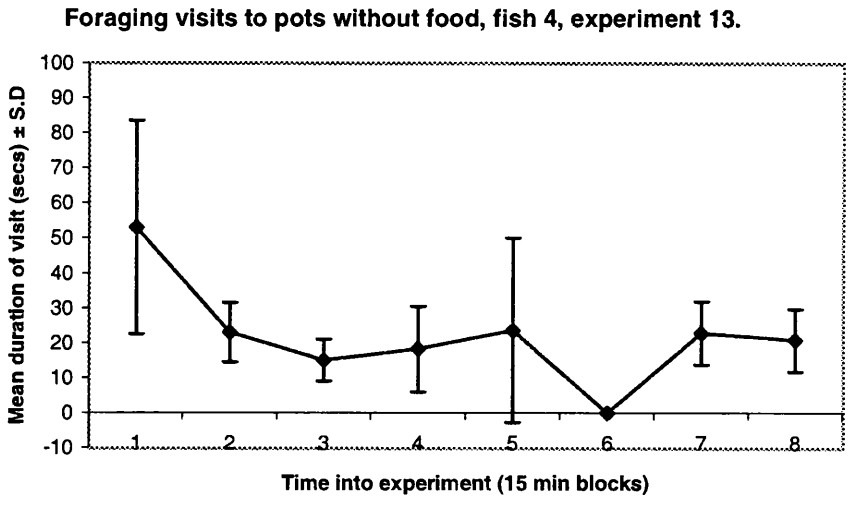


Figure 4.19c

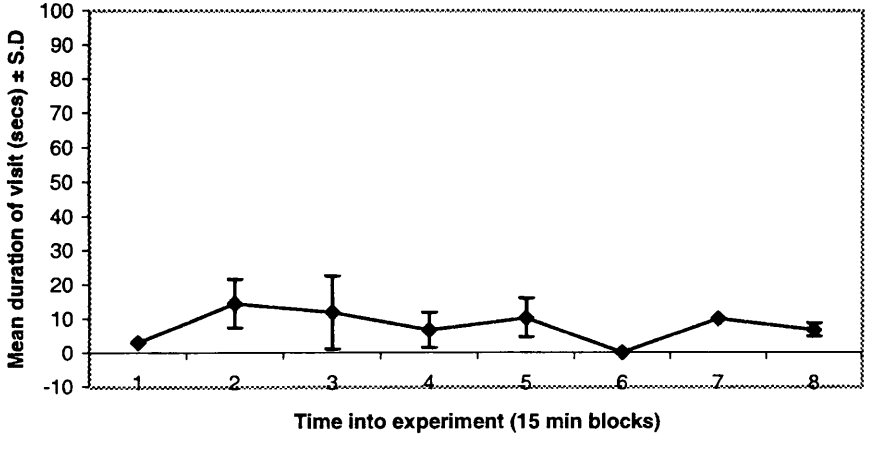


Figure 4.19d

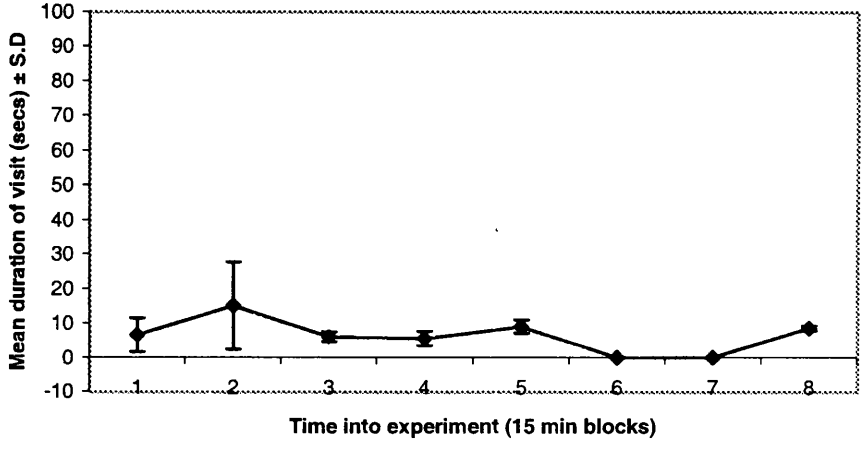


Figure 4.20

Mean duration of foraging visits for trials with a significant Chi-squared statistic with time into the experiment.

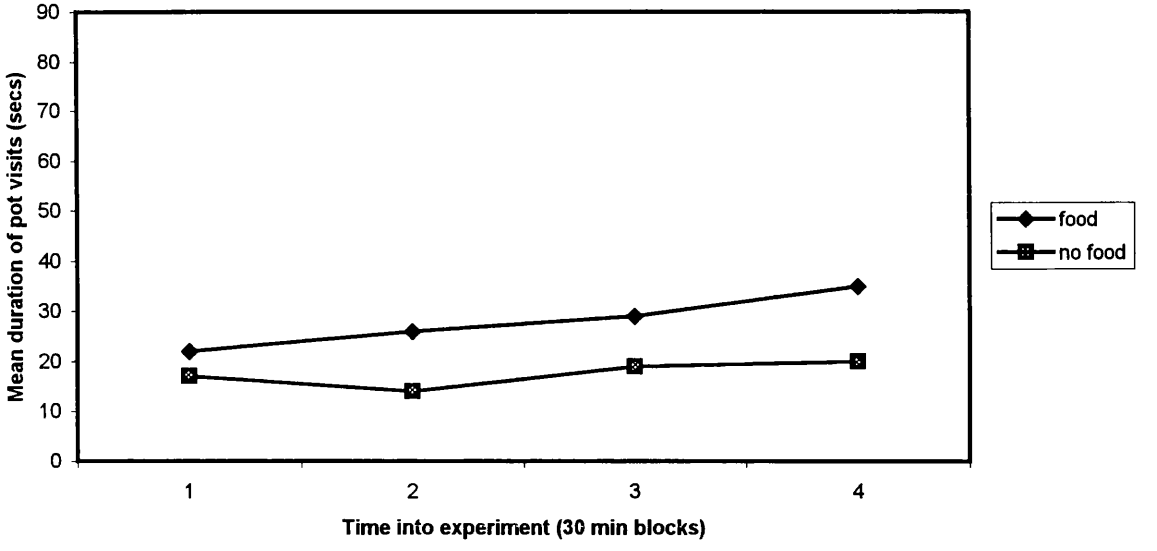
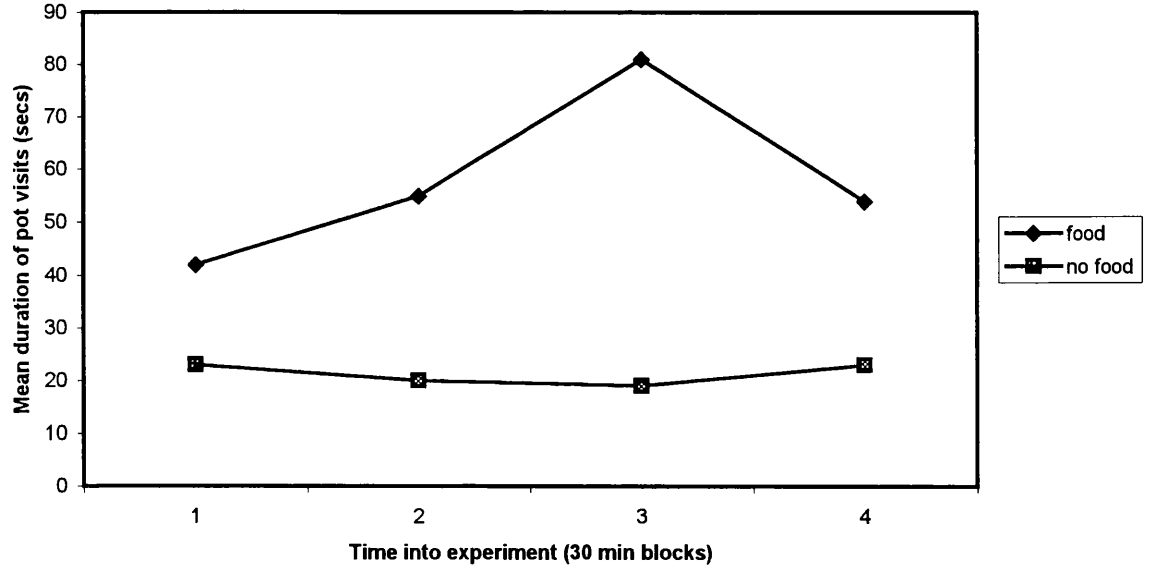


Figure 4.21

Mean duration of foraging visits for trials with a unsignificant Chi-squared statistic, with time into the experiment.



4.4 Discussion

Foraging behaviour

Through all the trials fish foraged actively, moving between pots quickly and foraging within pots intensively. This behaviour was quite different to that seen during the experiments described in chapter 5. When ruffe were observed foraging on *Asellus aquaticus*, in well lit tanks fish spent relatively long periods of time inactive, punctuated with short bouts of active foraging. In this study prey were not only concealed beneath the substrate, but were also relatively inactive compared to macroinvertabrates previously tested. The absence of light in the visual spectrum should also have made prey location more difficult for ruffe. In addition to bloodworms being confined to pots, these conditions would have made prey much more difficult to detect, and therefore it is not surprising that ruffe displayed very different foraging tactics than previously seen. Because prey were more difficult to locate and were at a low density, fish were forced into spending more time searching. This demonstrates that although ruffe can forage successfully in the absence of visual cues for concealed prey, it is more costly, requiring a greater effort than foraging for more active, conspicuous prey in the light.

This study has confirmed that ruffe are able to forage for concealed prey in the dark, using non-visual cues to detect prey; the ability to do this varied between fish. All fish foraged actively throughout the study period, but no pots were empty at the end of each trial, therefore foraging was not 100 percent efficient. The overall percentage of prey taken by all fish, over the six trials was 50 percent.

Fish used in this experiment had been trained to forage in pots under experimental conditions, prior to filming the experiments. Therefore they had also learnt that their presence in the arena was time limited, and so emerged from the living area soon after the partition was lifted. Time limits on foraging period could have influenced activity levels, increasing the time devoted to foraging.

Foraging decisions: discrimination between pots.

All fish visited pots with food more often than those without, although there is variation in frequency with which pots are visited by different fish between trials, with only some fish visiting food pots significantly more often than non-food pots. When examining the cumulative frequencies of visits to food and non-food pots, the only individuals with significantly different visit frequencies, are those for which the differences between observed and expected visits to pots increases as the trial progresses. These fish appear to learn which pots contain food throughout the course of the trial. Examination of all the data indicates that learning alone cannot be responsible for the discrimination between pots, as for most trials (the exception being fish 4, trial 12), pots with food in are visited more frequently than would be expected by chance. Therefore fish must be using distance cues when making decisions over which pots to visit. These cues could either be chemosensory or mechanosensory. It is not possible to determine from this experiment which of these cues, or whether it is a combination that attracts fish to food pots. However, these cues are not very effective, when considering that fish frequently visit pots containing no food. Therefore when foraging on small patches of concealed prey, visual cues may play an

important role. It is also possible that non-visual cues were less effective when foraging in the experimental tank than in the wild, for several reasons. The presence of pots may have interfered with lateral line sensitivity, reducing the ability to detect mechanosensory cues. Chironomids normally bury into soft sediments, which ruffe may find easier to forage in. Although the pots and the gravel inside each pot were cleaned after each trial, small traces of chemosensory cues from prey may have remained. This could lead to confusion for ruffe, if they were relying on chemosensory cues alone to detect the location of prey. An additional factor that could have influenced early foraging decisions in ruffe could be previous experience of the experimental arena. Some species of fish, such as the goldfish (*Carassius auratus*) can use visual cues and landmarks, when making foraging decisions based on previous experience (Warburton, 1990). It is possible that if ruffe remember the position of pots containing food from previous trials, this could influence the pots they visit early on in the experiment. Under natural conditions, remembering the location of profitable feeding patches could prove very useful, as it is unlikely that patches will be completely depleted.

Foraging decisions: bout duration.

All fish in all trials spent significantly longer foraging inside pots with food in than those without. No difference in the foraging duration between pots was seen, when foraging outside pots. There are two possible explanations for this, it could be that the outside pot visits are merely used as assessment of the resource potential of a pot. If this is true, it should take fish just as long to assess pots with food in than those without. The other possibility for this difference in outside and inside foraging visits

is that a form of sequential assessment occurs when fish are foraging inside pots. Assuming that fish find a source of food during foraging, they are likely to spend longer at that pot in order to exploit the food available (before moving on to another pot). Fish will also pick up more chemosensory and mechansensory cues from prey when they are near a pot containing food.

There must be a threshold duration, by which time if a fish has not detected the presence of prey or has not been able to feed, they will leave that particular patch and move onto another. However upon finding food, the fish will be induced to remain at that foraging site for long enough to exploit it further, until the amount of food found falls below the energy required to locate it (Charnov, 1976). In these experiments very few pots were totally emptied of food, although in some of the longer trials (not featured in this analysis), some of the pots had been completely emptied of all prey. Behaviour relating to the length of time a single foraging bout should last for each patch is subject to the food value of the patch combined with the foraging effort required to deplete the patch. This has been described in optimal foraging models. Marginal Value Theorem predicts that each foraging patch has a marginal value of food reward. Once the resource has been depleted to this threshold level, it is no longer optimal to forage in that area, and the forager should leave that patch and begin to search for new one (Charnov, 1976). Within this experiment, it is likely that the fish were forced to forage sub-optimally, as the number of food patches was small, and abundance of food within patches was low.

It is also possible that some form of assessment is used before a decision to forage inside a pot is made. Fish frequently forage around the outside of pots before making the decision to go into a pot, it is possible that during this phase fish are attempting to assess the resource potential of a pot, using this behaviour to detect cues, (possibly olfactory) from prey in the pot.

From these data, it seems that ruffe used one of two rather different foraging modes. In some sessions, visits to all pots are frequent and short (frequent foragers), whilst in others they are fewer and longer (long foragers). In frequent foragers, significantly more visits are made to pots with food and it seems that some sort of learning process is occurring. In long foragers, visits to pots with food are not significantly more frequent but are longer. This may have to do with overall hunger levels experienced by different fish, and clearly needs further study.

Related studies on the use of non-visual cues by ruffe

Other experiments on ruffe foraging behaviour have highlighted the importance of prey movements for ruffe in the location and capture of prey, by using a combination of sight and the lateral line. Previous studies have demonstrated that ruffe utilise their lateral lines when searching for prey in the dark, however none of these studies used prey items concealed beneath gravel. One study compared the foraging behaviour of both ruffe and yellow perch (*Perca flavescens*) in the dark, when foraging on free-swimming *Daphnia magna* and burrowing mayflies (*Hexagenia limbata*) secured in small tubes (to simulate burrows) (Janssen, 1997). Ruffe displayed faster swimming speeds and greater prey detection distances than perch, when foraging on both prey

species, indicating that they are better adapted to feeding in the absence of visual cues than yellow perch. Both fish species displayed very different methods of predation, with ruffe actively swimming, using a combination of pectoral fin flaps and tail beats, followed by gliding. Janssen suggests that ruffe use the gliding phase to detect mechanosensory cues from prey. Perch were able to use their lateral line in foraging, although they employed different methods of feeding depending on the prey species. When feeding on *Daphnia* they were inactive, eating individuals that swam into them, but were more active when foraging on mayflies, possibly because they were easier to detect.

Another study concerned with the foraging behaviour of both ruffe and yellow perch, examined slightly different aspects of the foraging behaviour in these fish (Savino and Kolar, 1996). This study used the prey species *Chironomus riparius*, because chironomids are know to feature in the diets of ruffe, (Ogle *et al.*, 1995). A soft substrate was used for the chirononmids to burrow into, and the foraging behaviour of both species were studied. Ruffe visited the feeding area fewer times than perch, but had longer foraging bouts while at the food containers. Ruffe also spent less time handling the prey before ingesting it, although both species had similar capture rates, demonstrating that ruffe may forage more efficiently than perch.

Conclusions and Further work

From examining the foraging behaviour of ruffe, it is possible to see how well they are adapted to feeding in the benthos for items like fish eggs and macroinvertebrates, which may be concealed amongst gravel substrates. To fully understand the foraging

behaviour of ruffe and their interactions between prey species, it is important to further investigate the use of non-visual cues in ruffe foraging by separating out chemosensory from mechanosensory cues. This could be carried out using a similar experimental design, substituting the live prey for both dead prey and moving artificial prey. Other prey species could also be used under similar conditions, to study whether ruffe utilise distinct foraging tactics when hunting for different types of prey, such as *Asellus aquaticus* or fish eggs, which are known to be important prey species for ruffe in Loch Lomond (Adams and Maitland, 1998).

Chapter 5

Foraging behaviour of ruffe (*Gymnocephalus cernuus*) and escape tactics of *Asellus aquaticus*

5.1 Introduction

Predator-prey interactions

Interactions between predators and their prey have been the subject of numerous studies, across a very wide range of species. The experiments described in this chapter examine foraging success in ruffe and how this is influenced by the behaviour, and reproductive status of their prey. As a background I review the literature on anti-predator adaptations (concentrating on invertebrate prey), prey selection in fish predators (concentrating on selection by size and reproductive status and the influence of habitat complexity) and previous studies of foraging in ruffe.

Predator avoidance strategies in invertebrates

Crypsis

Many studies have examined camouflage as a means of avoiding predation. The ability to blend into the surrounding environment is essential for many species. One example of a species that uses crypsis in the avoidance of predation is the sandhopper (*Talitrus saltator*; Ugolini, 1997). Sandhoppers are subject to predation from a variety of fish species; in this example the two-banded bream fish (*Diplodus vulgaris*) was used. Sandhoppers present on a background of light coloured sand were attacked and eaten significantly more that individuals present on dark sand, because they were much more visible against the lighter background. Activity was also important for sandhoppers in avoiding predation, with duration of survival increasing when fewer

movements were made. This demonstrates how simple methods of predator avoidance can prove effective against a visually foraging predator.

Chemical defences

The use of chemical defences in avoiding predation is widespread among invertebrates. Chemical defences can allow individuals to take advantage of profitable feeding areas whilst reducing their risk of predation. A study on the earthworm species *Dendrobaena octaedra* (Sav.) and *Dendrodrilus rubidus* (Sav.) examined the relationship of these two earthworm species with the red wood ant (*Formica aquilonia*; Laakso and Setälä, 1997). Both species of earthworm are abundant in the nest mounds of red wood ants, where they benefit from warm temperatures, moisture, abundant food sources and defence from predators. Under normal conditions ants are predatory on earthworms. However both these species secrete mucus that repels the ants. Although it does not prevent ants from being predatory on worms, it increases the time taken by ants to feed on them.

Chemical defence is important in the beetle *Tenebrio molitor*, which is subject to predation from a wide variety of invertebrate and vertebrate predators. This species respond to an attack by everting their abdominal glands, which secrete chemicals that are known predator repellents. Chemical defence was significantly reduced in individuals infected with cysticercoids of the tapeworm *Hymenolepis diminuta*. As a consequence of this infected beetles were more prone to predation by rats, because they were more palatable than uninfected conspecifics (Blankespoor *et al.*, 1997).

Some species are able to render themselves unpalatable by obtaining certain compounds from their food sources. Examples of this can be found in butterflies belonging to the families Danainae and Ithomiinae along with the Arctiidae moths. These species obtain pyrrolizidine alkaloids (PAs) from plants belonging to a number of genera (Trigo *et al*, 1996). Studies on Ithomiinae butterflies have shown that they obtain PAs from a variety of plant sources, including buds and withered leaves, and that PAs play an important role in chemical defence for Ithomiinae butterflies against the spider *Nephila clavipes*. These spiders will often liberate chemically defended butterflies from webs without harming them. Ithomiinae butterflies also display warning colouration, which serves as a defence from birds and other visually mediated predators (reviewed by Trigo *et al*, 1996).

An indirect method of chemical defence is Batesian mimicry, where palatable species mimic the appearance of unpalatable species, in order to avoid predation by visual predators. This phenomenon is found in several species of butterfly, and is often sex-biased with only females adopting mimicry. A study that examined the frequency of beak damage (by birds) on male and female butterflies belonging to the families Papilionidae, Pieridae (palatable) and the unpalatable Danaidae, found that beak damage was present on significantly more females than males in all of the families studied (Ohsaki, 1995). This suggests that birds selectively attack females, possibly because carrying eggs reduces flight performance making them easier to catch. Females also hover above oviposition sites, which may also increase vulnerability to predation. The frequency of beak marks was also studied in *Pachliopta aristolochiae*, an unpalatable butterfly and *Papilio polytes*, a species that mimics the former.

Mimetic females had significantly fewer beak marks than non-mimetic females, and males (that do not exhibit mimicry) had a similar number of beak marks to unpalatable females and mimics. This demonstrates that batesian mimicry is an effective form of predator avoidance, although it is dependant on the density of mimics in the population remaining relatively low (Ohsaki, 1995).

The previously discussed methods of predator avoidance incur relatively few costs to the individual. There are some methods of escape, however, that can prove very costly.

Autotomy

Many species shed limbs as a means of escaping predation, (autotomy). This is a costly way of avoiding predators, and so is mainly used when under immediate threat, and as a last resort. Damselfly larvae (Odonta) are known to autotomise caudal lamellae in order to escape predation and cannibalism. This is a useful tactic to avoid immediate threat, but results in a number of disadvantages. To study the implications of lamellae autotomy for damselfly larvae, an experiment was conducted using *Lestes sponsa* (Stoks, 1998). Some individuals were induced to autotomise their caudal lamellae whilst others remained intact, they were then subject to predation by both conspecifics and *Notonecta glauca* (common backswimmer). It was found that the absence of caudal lamellae increased the susceptibility to predatory attack from both conspecifics and *N. glauca*, reduced hunting success was also observed. This was caused by a reduction in swimming speed and mobility, making predator avoidance

and hunting more difficult for individuals without lamellae. Although lamellae autotomy is a useful adaptation against predation, there are a number of costs.

Spiders are also known to autotomise legs in order to escape predation. A study was conducted on the spider *Kukulcania* (*Filistata*) *hibernalis*, and the effectiveness of leg autotomy from evading predation by the scorpion (*Centruroides vittatus*) and the centipede (*Scolopendra polymorpha*). It was found that leg autotomy was a highly effective defence against predation by scorpions, but useless against centipedes because of the speed at which centipedes bite and poison their prey (Formanowicz, 1990).

Thanatosis

Some species use a strategy of feigning death (thanatosis) to escape predation. This has been observed in the cricket *Gryllus bimaculatus*, which will adopt thanatosis when its forelegs and pronotum are held (Nishino and Sakai, 1996). Whilst in this state, individuals increased their heart rate and decreased their respiration rate, when remaining rigid. Although it was not tested in this study, it is likely that the adoption of this behaviour helps individuals to escape from a range of predators.

Foraging strategies in fish

Selection of prey on a basis of gender and reproductive category

Certain life-history traits and behaviours can predispose prey to a higher risk of predation. Behavioural and morphological traits associated with reproduction can

increase the susceptibility of one gender over another to predation. This is commonly seen as predators feeding selectively on a particular gender or reproductive category. Many species are seen to feed selectively on males, this may simply be a result of increased encounter rates with males that are searching for mates, rather than actually seeking out and choosing males specifically. This is seen in the male tick-tock cicada (*Cicadetta quadricincta*) (Gwynne, 1987). In this species males actively seek out females by making a series of flights separated by periods of perching, when males call to mates, the female cicadas respond by calling back, males use these calls to locate females. The high mobility of the males made them more prone to predation by web spinning spiders, because of spending more time flying than females. This is a case where predators were not selecting for males, but that the mating behaviour of the males was increasing their risk of predation.

In other cases predators can be seen to actively select one gender or reproductive category over another. The susceptibility of water striders (*Gerris buenoi*) to predation by backswimmers (*Notonecta kirkii*) was studied (Rowe, 1994). Mating behaviour in this species involves a high level of female harassment by males, and can increase the likelihood of predation, through creation of water disturbance that attracts predators. During mating the female carries the male, and as a result has a reduced mobility, although these females actively seek out shelter on floating vegetation, they are still twice as likely to be eaten by backswimmers when mating, then when they are alone.

Some species of fish are known to forage selectively on gravid females. This can be seen in the waterflea (*Daphnia* sp.; Mellors, 1975), where fish selectively fed on gravid females, made more conspicuous to visually foraging predatory fish by the presence of ephippial eggs. In response to this selection pressure, eggs in this species have become resistant to digestion. A similar pattern is also seen in the copepod *Eudiaptomus gracilis* when under predation pressure by roach (*Rutilus rutilus*; Svensson, 1997). Roach were fed on a population of copepods in an enclosure, at the end of the experiment there were significantly fewer females carrying eggs present in the fish enclosure than in the enclosure where fish were not present. Those carrying eggs had significantly smaller clutch sizes in the fish enclosures, it is thought that carrying eggs increases conspicuousness, and the more eggs in a clutch the more visible an individual is. Roach also selected prey by size, consuming larger male and female copepods. This selection for larger individuals was more marked in males than females, because the presence of eggs is a more conspicuous signal than large body size for foraging roach, and in this copepod species body size is not related to clutch size.

Size selection of A. aquaticus by perch

Previous studies have examined the importance of an *A. aquaticus* population in the diet of a perch (*Perca fluviatilis*) population in the wild, (Rask and Hiisivouri, 1985). This study found that diet compositions in different size and age categories of perch varied. The smallest size group of fish 6.9-9.0cm (1-2 years in age) ate few *A. aquaticus*, preferring zooplankton. Larger fish ate *A. aquaticus* in higher numbers, with the maximum consumption occurring in the size range 11.0-12.9 cm (4-6 years

in age), above this size perch fed increasing on larger invertebrates such as caddisfly larvae (Trichoptera) and the larvae of dragonfiles and damselfiles (Odonata). Perch fed on all size ranges of *A. aquaticus*, although newly released juveniles only appeared in the fish diet at sizes ranging from 2.7 to 4.0mm, when at first emergence they are present in the population at sizes between 2.0-2.4mm. Perch did appear to select for larger sized *A. aquaticus*, because large individuals remained common in the diets of perch even when they were relatively rare in the population. This study also stresses the importance of mobility in the *A. aquaticus* population and the scarcity of vegetation for shelter, in making them a particularly important prey species for visually foraging predators such as perch.

Habitat complexity

Foraging efficiency of a predator, and predator avoidance in prey species is strongly influenced by habitat complexity. Habitat complexity is a term used to describe the structural complexity of a habitat, which is determined by including the presence of macrophytes, and the type of substrate found on the bed. Habitats with a high structural complexity may consist of large densities of submerged vegetation, and a bed with a gravel substrate, this habitat provides many potential refuges for prey and therefore makes foraging difficult for visual predators. Habitats with low complexity, can be seen in man-made drainage canals consisting of a flat concrete bed and banks, but are also present in oligotrophic lakes, where a lack of dissolved minerals reduces the presence of plant life. A study on the predation of *Asellus aquaticus* by a visually foraging predator, the perch (*Perca fluviatilis*) in a small Finnish lake suggests that scarcity in vegetation contributes to the high levels of perch predation on *A.*

aquaticus, (Rask and Hiisivuori, 1985). Because *A. aquaticus* are a highly visible prey species, they rely heavily on submerged vegetation to provide cover from fish predators.

It is generally accepted that an increase in habitat complexity is beneficial to prey species, as it makes prey detection more difficult. This is not always the case, and whether high levels of complexity are advantageous or not, is dependant on which species of predators and prey are involved, and the methods employed in predation and predator avoidance. A recent study has highlighted the influence of habitat complexity on the behaviour of a prey species with two different predators, (Flynn and Ritz, 1999). In experimental tanks containing open water and differing degrees of habitat complexity, the behaviour and habitat use of the mysid (*Paramesopodopsis rufa*) and its predators the seahorse (*Hippocampus abdominalis*) and Australian salmon (*Arripis trutta*) were studied. Mysids spent all their time swarming in open water. When habitat complexity was increased swarms decreased in size but increased in number. The influence of changing habitat structure on predator attack and success rates was also recorded. They found that although the attack rates of juvenile Australian salmon decreased with increasing structural complexity, the percentage of successful attacks increased. Juvenile salmon spent all their time in open water, and employed a lunging tactic when catching prey. Seahorses used very different tactics, spending almost all the time in vegetated areas of the tank, using ambush predation to catch individuals on the periphery of the swarm, the rate of successful attacks also increased for seahorses with increasing habitat complexity. It therefore appears that species which use swarming as an anti-predator tactic, are more

vulnerable in habitats where vegetation is at high densities, because this breaks up large swarms into smaller more numerous ones. Swarms aid predator evasion by a combination of different factors. These rely on the swarm being large, to detect the presence of predators more rapidly and to cause confusion for predators; by breaking swarms into smaller units these abilities are reduced.

Influence of turbidity on predator-prey relationships

Other environmental factors can effect the ability of visual predators to forage effectively, one of these is the turbidity of water. Many habitats are turbid, which greatly reduces visibility. A study on the influence of turbidity on predator-prey interactions between fat-head minnows (*Pimephales promelas*) and yellow perch (*Perca flavescens*), showed that in turbid water fat-head minnows took more risks when foraging in the presence of a predator than when in clear water (Abrahams and Kattenfeld, 1997). Although water turbidity did not influence predation rates by yellow perch, it did reduce prey size selection. In clear water smaller individuals were selected. However in turbid water no size selection occurred. Abrahams and Kattenfeld suggest that in turbid water the effectiveness of anti-predator behaviour is reduced, because when in clear water larger individuals are better able to escape predation.

Previous studies on ruffe diets

The ruffe is a benthic fish, which feeds on a variety of macroinvertebrate prey. Although there have been numerous studies on the diet of ruffe, relatively little is known about the foraging behaviour of this species. Most studies involving ruffe

foraging behaviour have centred on foraging efficiency under different conditions and competitive ability (Bergman, 1987; Bergman, 1988).

Studies on the diets of ruffe have demonstrated that they eat similar species across their distribution. In Loch Lomond over the winter months, when benthic macroinvertebrates are in relatively short supply, ruffe mainly feed on fish eggs and fry, (Adams and Tippett, 1991), although *A. aquaticus* are also important. During the summer when *A. aquaticus* are plentiful, they are very important for ruffe.
The diets of a population of introduced ruffe in Lake Superior have been studied, (Ogle *et al.*, 1995). Small, age 0 ruffe fed on small invertebrates, including copepods, cladocerans and chironomids. Adults feed mainly on chironomids and crustaceans, whereas large individuals over 12cm included other prey species such as caddis fly larvae.

Aims

We were interested in learning more about how ruffe forage, but also wanted to examine the behaviour of *A. aquaticus* in the presence of ruffe, and the effect of shelter on the behavioural interactions of these species (relating to Chapters 2 and 3). The experiment consisted of three main areas for investigation:

- To examine the behaviour of ruffe foraging for *A. aquaticus* in the absence and presence of shelters (using data collected from behavioural observations).

- To investigate the predator avoidance tactics employed by *A. aquaticus* with and without shelters (using data collected from behavioural observations).

- To ascertain whether ruffe display prey selectivity, on the grounds of size or reproductive status.

- To examine the stomach contents of a ruffe, after feeding to satiation with *A. aquaticus*.

5.2 Materials and Methods

Collection and maintenance of study animals

Ruffe (Gymnocephlus cernuus)

Ruffe are a common species of benthivorous fish in Loch Lomond, Scotland and were collected using a bottom trawl at different occasions throughout spring and summer 1998. These individuals were initially maintained in a large stock tank (at the Universities of Glasgow and Stirling Field Station, Rowardennan, Loch Lomondside, Scotland), which had a constant flow of loch water running through it. Prior to the experimental runs, individuals were moved to aquaria within Glasgow University. Throughout both these periods, individuals were fed frozen chironomid larvae on a daily basis. Prior to using each individual for the behavioural experiments their diet was switched to live *Asellus aquaticus*, which involved adding a few individuals to the experimental tank every other day. This was used as a period to become settled into the experimental tank, and to become used to feeding on live prey items.

Asellus aquaticus

Asellus aquaticus is a freshwater isopod common in the diets of many fish species in Loch Lomond. Attempts had been made over the years 1996 to 1998 to catch *A. aquaticus* from sites within Loch Lomond. Despite catching a few individuals (approximately ten), I was largely unsuccessful. Instead I used two main sites, the Forth and Clyde Canal, at Bishopbriggs (O.S. Landranger 64; 604,716) and a pond in Hamilton, (O.S. Landranger 64; 724,566). Invertebrates were caught using a dip net, to collect them from both the benthos and amongst macrophytes, *A. aquaticus* were

removed from the nets and transported back to aquaria in water-tight storage pots. Individuals were kept in stock tanks according to which site they were collected from. All genders were kept together in the stock tanks, and were provided with decaying leaves as a food source.

Experimental tank design

All the experiments were conducted within a tank (figure 5.1) 60cm X 30cm, and 30cm deep, which was raised off the floor to prevent vibrations from people walking around disturbing the fish. The tanks were filled with washed builder's sand to an approximate depth of 2cm. This was to provide a substrate, which would enable *A. aquaticus* to walk around the tank more easily, but it would not be a source of refuge. This sand also helped the *A. aquaticus* to stand out from the background, making them easier to see when making observations. This also however had the effect of making them more visually conspicuous for the fish predator, when in the wild it is more likely that the background would be darker and contain shelters for prey.

Experimental procedure for experiments with and without shelters

Each fish was introduced to the experimental tank several days prior to the start of experiments (see table 5.1 for details of fish and repetitions). During this time the fish were provided with live *A. aquaticus* every two days, to become used to feeding under experimental conditions. After a maximum of a week in the experimental tank, the studies began.

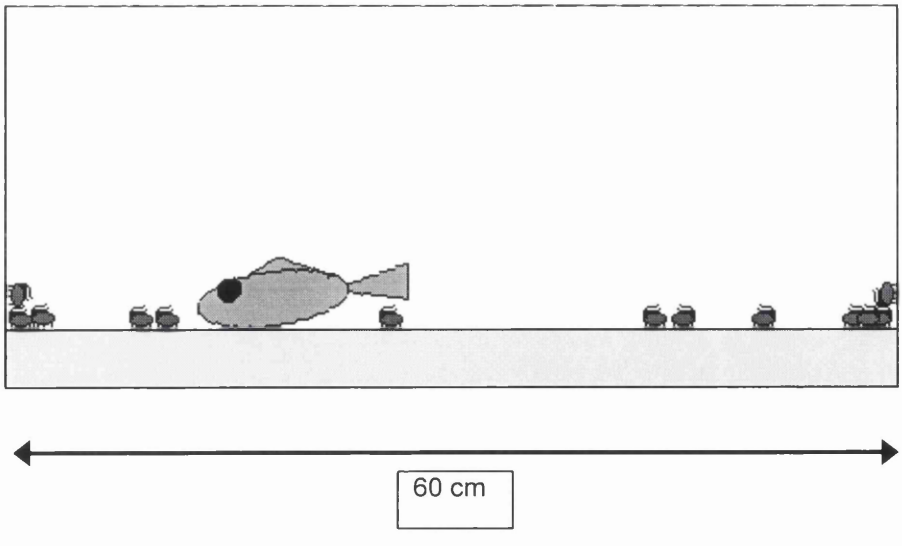
Table 5.1

Details of the fish used in the experiments and the number of repetitions

Fish number	Experimental conditions	Number of repetitions
1	shelters present	2
4	shelters absent	4
5	shelters absent	4

Figure 5.1

Diagram of experimental tank set-up



Before each experimental run, *A. aquaticus* were selected from the stock tanks on the basis of gender and reproductive status. Attempts were also made to select a wide range of sizes of each reproductive category, although this was often difficult to do due to the life history of this species. Larger individuals die off as the spring and summer progress, leaving a smaller size variation from mid-summer onwards. Each *A. aquaticus* was measured and their reproductive status and gender recorded prior to each experiment. Thirty individuals were selected as prey for each experiment. These were comprised of roughly 10 males, 10 brooding females, 10 post-brooding females, and 5 mate-guarding pairs. The exact numbers used of each reproductive category depended on their availability, which also varied throughout the year. The measuring of *A. aquaticus* was carried out using a piece of graph paper (with millimetre squares) inside a clear plastic (waterproof) folder pocket.

A. aquaticus were added to the experimental tank from behind a black plastic curtain, to prevent the fish from being startled. From behind this, observations were made on the behaviour of both ruffe and *A. aquaticus*.

Attempts were made to record the time at which each prey item was eaten. This was made difficult by the dim lighting. Fish also disturbed the substrate when foraging, further reducing visibility. Often foraging consisted of short periods with a rapid succession of attacks on prey, making it very difficult to record the timing of all attacks.

The experiment was run for up to one hour or until roughly half the *A. aquaticus* had been eaten. The idea behind this was to allow the fish to eat enough individuals to get an idea of whether it was selecting certain prey sizes or categories, without letting it eat all the individuals present, to allow statistical comparison of eaten and uneaten prey. At the end of the experiment all the remaining *A. aquaticus* were collected, identified for reproductive category, and measured.

Experiments with shelters

Initially a few experiments were carried out using shelters in the tank, which the prey could utilise as shelter. This was done to gain an idea of how ruffe would react when faced with the problem of prey in shelters, and to see whether they were able or willing to forage amongst macrophyte like shelters for prey.

I found the results of this interesting. However, I did not repeat this experiment many times, because almost all the prey took refuge within these shelters. Therefore I was unable under this experimental design to study the prey selectivity of ruffe, as the fish simply ate everything that was not in the shelters.

Prey selectivity investigations

Data were collected on the number of different sized *A. aquaticus* eaten, and which reproductive category they belonged to. Due to difficulty obtaining sufficient repetitions for different fish, all the data from each fish has been pooled in order to carry out the necessary graphs and statistical analysis.

Examination of the stomach contents of a ruffe

A fish that had previously been trained to feed on *A. aquaticus*, and had been used for experiments on prey selectivity by ruffe was selected for this experiment. The fish was starved for two days in order to ensure that it would feed. *A. aquaticus* which had previously been caught in the Forth and Clyde Canal at a site near Bishopbriggs, Glasgow, were removed from stock tanks and were added to the experimental tank containing the starved fish. In all 92 individuals were added to the tank, and these were left for 2½ hours. After this time the fish was removed from the tank and was sacrificed using an overdose of benzocaine. The fish was then dissected, photographs were taken and a record made of the stomach contents of the fish. Measurements were taken of the prey removed from the stomach, and their gender was recorded.

5.3 Results

Ruffe foraging behaviour

Ruffe remained quite still during most of the experiment. They frequently adopted the same position, with their heads close to the substrate, and their tails slightly elevated. I will refer to this as the resting position from herein. They remained motionless like this for much of the experiment, only occasionally swimming around the tank. Prey movements appeared to be the principle stimulus for foraging fish during these experiments. It is likely that these movements provided both visual and mechanosensory stimuli for foraging ruffe. Often when a fish was in the resting position, prey would 'walk' near or under the fish. In situations when prey were moving in close proximity to or under its head, the fish would make a rapid head movement towards the stimulus usually going on to catch and eat the prey. In situations such as these it is unlikely that the fish could utilise visual cues, and instead may have been reacting to mechanosensory cues from the water disturbance or from prey movements directly. Ruffe did not react in the same way when moving prey occurred further along the body. This supports the use of mechanosensory stimuli, as there are many more sensory canals around the head of ruffe than along the body, (Disler and Smirnov, 1977; Gray and Best, 1989).

On other occasions, ruffe appeared to be utilising visual cues in prey detection and capture. When ruffe were swimming slowly close to the substrate, *A. aquaticus* would often run away quickly, reacting to the presence of the predator, the fish would respond by suddenly turning around and swimming after the prey item before

attacking it. Attacks made when moving at speed were not always successful, as the resulting water disturbance created by moving ruffe sometimes allowed prey to escape. Occasionally large individuals would escape during handling. The behaviour of the foraging fish suggested that at close range mechanosensory cues are important, whereas visual stimuli are important in locating prey when not in direct contact with it.

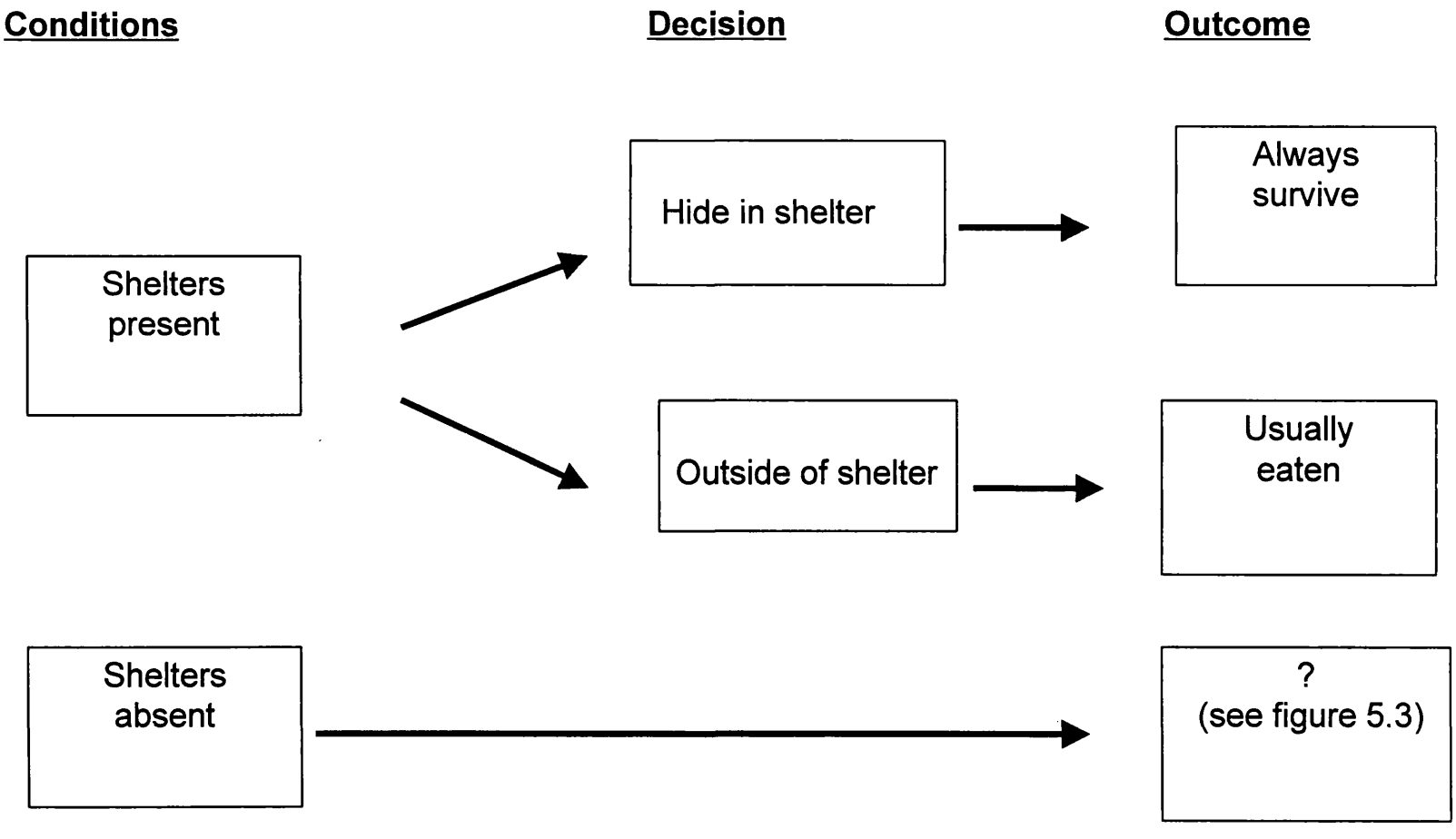
Foraging rates of the fish in the experiments were quite erratic, spending short periods actively foraging, but long periods stationary. Fish also had very different reactions to prey at different points in an experimental run, sometimes actively foraging, and other times seeming uninterested in prey. It is possible that this may have been influenced by how recently a prey item had been ingested, but it is not possible to ascertain whether fish in these experiments showed different foraging strategies or intensities as time progressed.

Observations of *A. aquaticus* behaviour

In the presence of two artificial shelters almost all the individuals present took shelter. The flow diagram (figure 5.2) illustrates the advantages of using shelters in predator avoidance. Under these circumstances, ruffe were seen to be more persistent in foraging attempts made on the few individuals that were outside of the shelters. The ruffe were unable to forage amongst the shelters, and often waited just under the shelters or beside them waiting for individuals that strayed out of the shelter, when this happened the fish rapidly caught them.

Figure 5.2

Flow diagram showing the behavioural decisions available to prey, and the possible outcomes when under two different conditions



Within the study three distinct anti-predator tactics were utilised by *A. aquaticus* in the absence of shelters (see figure 5.3). One of these was to simply avoid coming into contact with the predator, and involved individuals aggregating into small groups in the corners of the tank. This prevented the fish from both detecting and catching the prey. Because space in these corners was limited, most individuals rather than being in the tank corners, instead moved along the edges of the tank. This did not confer safety from predation, but made them more difficult to catch.

When directly faced with the threat from a predator, individual *A. aquaticus* generally used one of two strategies, (see figure 5.3). The first involved running away from the fish, and was often employed when an individual was some distance from the tank sides. This escape tactic was used relatively infrequently and was rarely successful, because the increased movement often attracted greater attention from the fish. The second strategy was the reverse of the first and involved remaining completely still. This will be referred to as 'freezing', and often proved a successful means of escape. Often the fish would be attracted to a moving individual who sensing the threat would freeze, once the prey had stopped moving the fish would search for it, mostly failing to locate it (even when the fishes mouth was directly above the prey). When this occurred in the centre of the tank, once the predator stopped searching and had left the immediate area, the *A. aquaticus* would often run to a side or corner of the tank.

Figure 5.3

Flow diagram illustrating the outcomes of predator-prey interactions, detailing the responses of predators to prey behaviour in the absence of refuges.

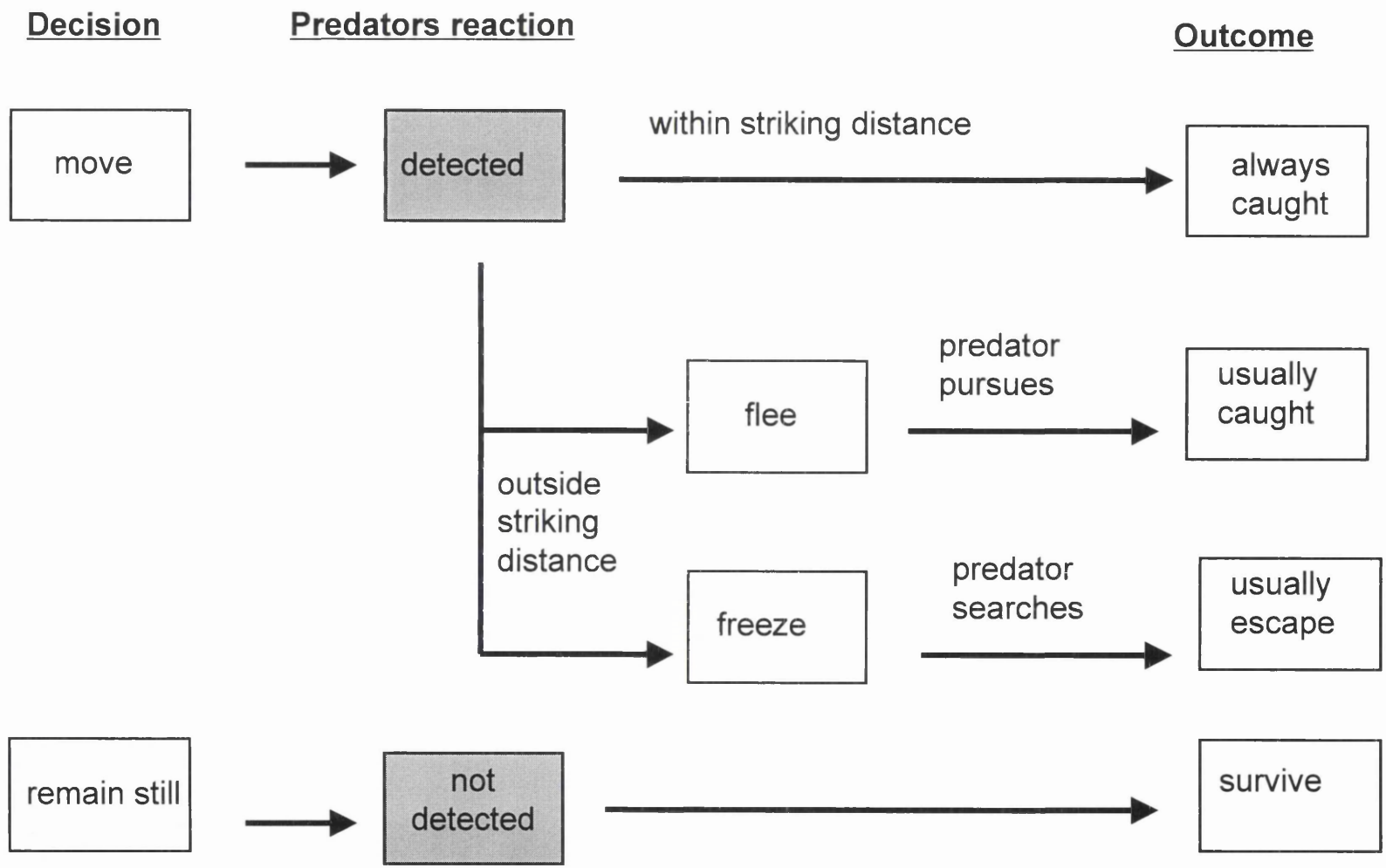


Figure 5.4

Proportion of *A. aquaticus* size classes eaten by ruffe

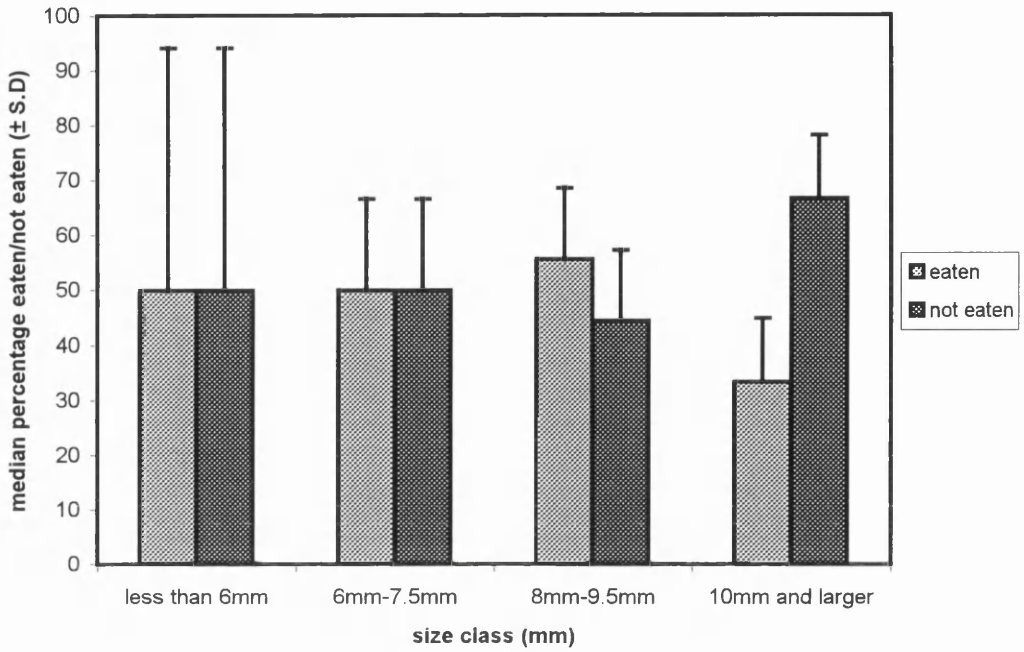
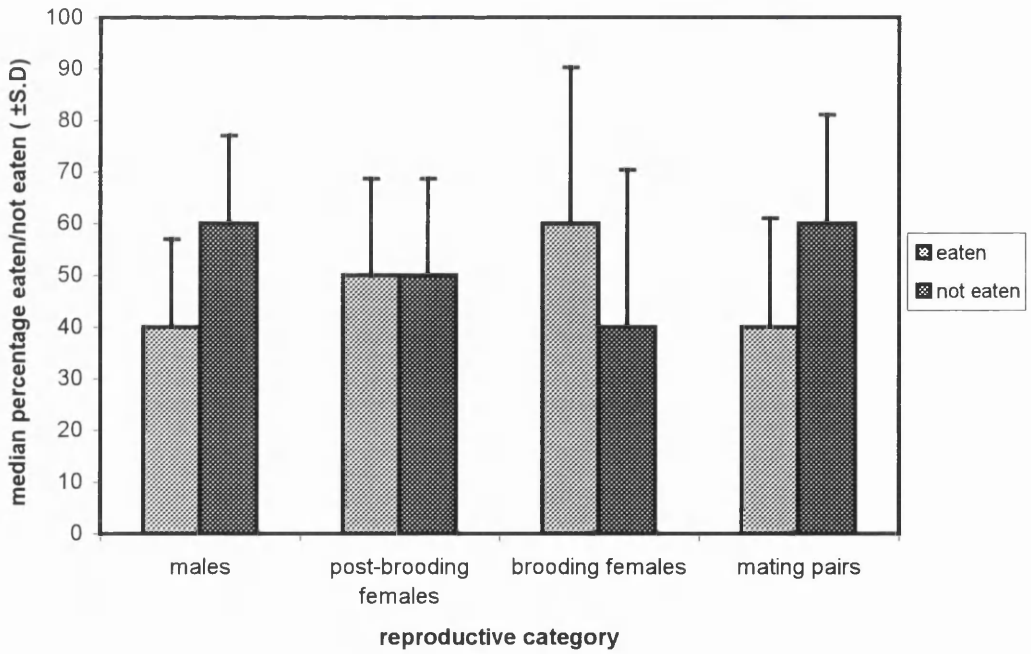


Figure 5.5

Proportion of different reproductive categories eaten by ruffe



Prey selectivity by ruffe

The data collected show that ruffe do not display any prey selectivity, as no differences exist in the numbers eaten and surviving. Figure 5.4 shows the proportion of different size classes eaten. It does appear that there are more survivors in the largest size class (10mm and over). However a Chi-squared test confirmed that ruffe do not select prey according to size (d.f = 3, χ^2= 5.0, P= 0.172). Similar results were found when the data on reproductive categories were examined. Figure 5.5 shows that there is little difference between the proportion of individuals eaten in each reproductive category. Analysis by Chi-squared test found a marginal but not significant difference between the number of individuals eaten and those surviving in different reproductive categories, (d.f = 3, χ^2 = 6.83, P= 0.077).

Examination of stomach contents of ruffe

The fish weighed 15.65g and had a standard length of 96mm. It was female with ovaries weighing 1.68g. The stomach appeared very full (Figure 5.8), and in total 39 *A. aquaticus* were removed during dissection. Measurements were not made of the available *A. aquaticus* prior to the experiment, although a record had been kept of their gender (Table 5.2).

The individuals removed from the stomach (Figures 5.8 to 5.9), were measured and their genders ascertained (although this was difficult in some cases). The fish ate a wide variety of prey sizes (figure 5.6); there were very few (2 individuals) prey larger than 10 mm available, which is reflected in their absence from the stomach. Most

individuals eaten by the fish were in the second size class, which was the most frequently occurring size category available. There did not appear to be any change in the prey sizes eaten throughout the experiment, as the last 2 individuals removed from the anterior section of the stomach were very different in size (a 4mm juvenile and 9mm male), shown in Figure 5.10. All the *A. aquaticus* removed from the stomach are shown in Figure 5.11.

Males were the most abundant reproductive category available, and approximately half were eaten (Figure 5.7). Proportionately smaller numbers of the other reproductive categories were eaten, but this could just be a product of reduced encounter rate due to the lower frequencies of other reproductive categories that were available.

These results further confirm that ruffe in this experiment were not selecting prey according to size or reproductive category.

Table 5.2

Number of *A. aquaticus* from different reproductive categories used in the experiment

Reproductive category	**No. in experiment**
Males	60
Brooding females	1
Post-brooding females	7
Juveniles	23
Mating pairs	1
Total	92

Figure 5.6

Frequency at which different size classes of *A. aquaticus* occur in a ruffe stomach

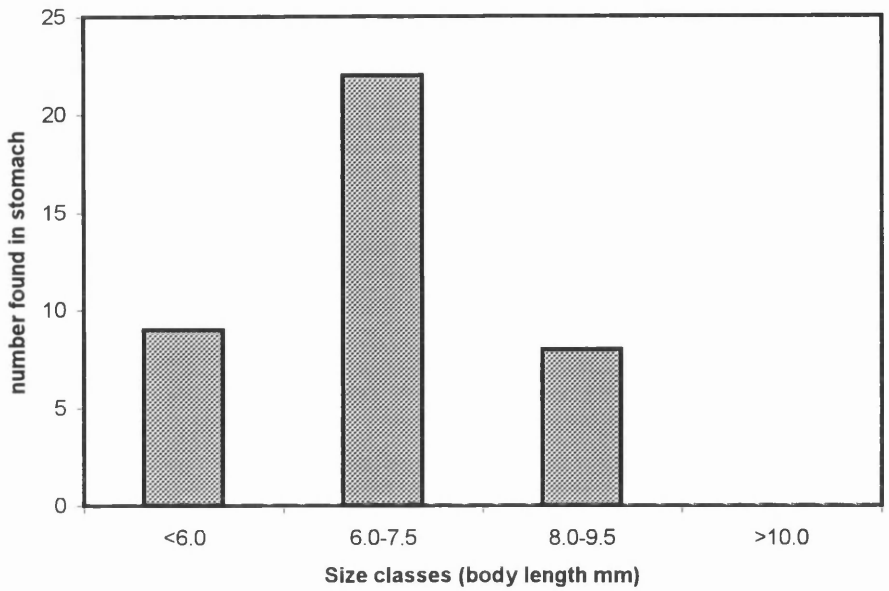


Figure 5.7

Proportion of available prey from different reproductive categories eaten

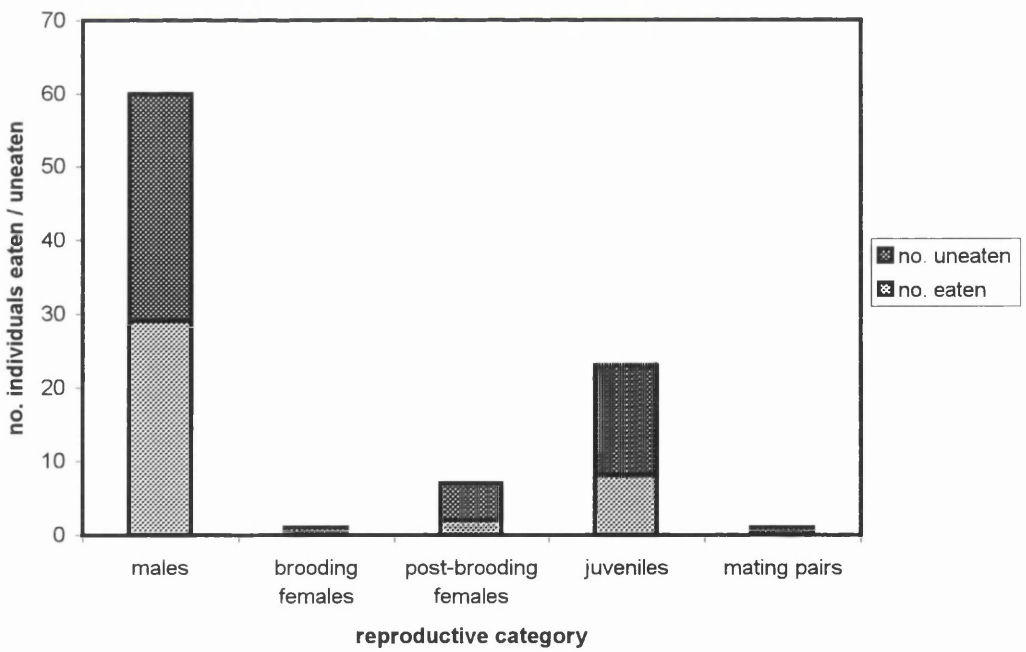


Figure 5.8

A. aquaticus packed into the stomach of ruffe

Figure 5.8a

Stomach slightly open

Figure 5.8b

Stomach opened wide

Scale: 1.9cm =1cm

Figure 5.9

Ruffe with some of stomach contents removed

Scale: 1.1cm = 1cm

Figure 5.10

Last 2 *A. aquaticus* removed from the anterior portion of the stomach

Scale: 1.9cm = 1cm

(Largest; male, 9mm; smallest, juvenile, 4mm)

Figure 5.11

A. aquaticus removed from the stomach of a ruffe

Figure 5.11a

A. aquaticus removed from posterior portion of stomach

Figure 5.11b

A. aquaticus removed from anterior portion of stomach

Scale: 1 small square on background = 1mm

5.4 Discussion

All individuals face a dilemma when foraging; to get the best energy return from the available prey whilst using as little time and energy as possible. In order to forage optimally, a variety of choices must be made depending on a wide range of factors; these may include prey species, prey size, prey density, habitat type and degree of hunger.

Ruffe displayed little foraging activity, when compared with previous experiments, under different conditions (Chapter 4). They foraged effectively for *A. aquaticus*, despite the low levels of activity, which may indicate they were foraging efficiently.

Prey selectivity

All the fish used in this experiment were fully mature fish. Although prey size can be a limiting factor for predators, it appears that the sizes of *A. aquaticus* used in this experiment did not exceed that which ruffe could forage effectively on. It is likely that if smaller fish had been used, differences may have been seen in the frequency with which some size classes were eaten over others. If smaller fish had been used, it is possible that *A. aquaticus* size could have become a limiting factor in prey selection. Prey handling time is known to increase with increasing prey size, and the relative size differences between small fish and large *A. aquaticus* will make capture and handling of prey more difficult. (Hart and Gill, 1992). From observations made during the experiments fish did occasionally show some difficulties in handling

particularly large prey items, sometimes spitting them out before catching them again. On a few occasions these individuals actually managed to escape.

Prey size and gut fullness

Prey size is known to be an important factor for predators when selecting prey items. Larger prey sizes can increase the handling time, and therefore can reduce the efficiency of foraging. Although prey size was not seen to be an important factor in prey selection for the fish in this experiment, other studies have highlighted its importance. Previous studies using *A. aquaticus* as the prey species have found prey size and gut fullness to be important factors when selecting prey. Hart and Gill, 1992, studied the influence of these factors on *A. aquaticus* selection by the three-spined stickleback (*Gasterosteus aculeatus*, L.). They found that fish larger than 45mm consumed larger *A. aquaticus* in greater quantities than smaller fish. All the fish in our experiment were between 80 and 100 mm in length, and therefore it is perhaps not surprising that no size selection of *A. aquaticus* was seen. Gut fullness was shown to play a role in selection of prey (Hart and Gill, 1992), with fewer large *A. aquaticus* being accepted as gut fullness increased, observing that fish became more selective towards prey as gut fullness increased.

Gut fullness is not likely to have played an important role in my experiment, as fish were only allowed to eat up to 15 prey items before the experiment was terminated. A brief run with one fish allowing it to forage for over 2 hours, showed that it had eaten a whole range of sizes, and it is possible that the fish may have been able to eat more. The last two individuals removed from the fish (which had not yet reached the

stomach) were of very different sizes, penultimate 4mm (juvenile) and last 9mm (male) see figure 5.10.

One species of Isopod (*Lirceus fontinalis*) has been shown to exploit the prey size constraints of one of its predators to its advantage, (Sparkes, 1996). Populations present in streams that have no fish predators, where the largest predators are salamander larvae, mature at larger sizes as a strategy of avoiding predation. Populations in streams where fish predators are present mature at small sizes and therefore remain at risk of predation.

Later studies have also highlighted the importance of gut fullness on prey selectivity by predators. A study on prey selectivity and handling time was carried out on four different aquatic species, Hirvonen and Ranta, (1996). They studied prey selectivity by four different species, two species of dragonfly larvae, *Aeshna juncea* and *Leucorhinia dubia*, along with the ten-spined stickleback (*Pungitius pungitius*) and the smooth newt (*Triturus vulgaris*). They found the same trends in all four species, where individuals tended to include fewer large and medium sized prey items as the foraging bout progressed. They linked this to the fact that as time went on, the handling time of larger prey increased, therefore making it less profitable to select larger individuals, whilst the handling time of smaller individuals remained the same. Perhaps then it is no surprise that I saw no size selectivity in ruffe, as I used large fish in relation to prey size, and only allowed them to feed on a small number of prey relative to the capacity of their stomachs.

Reproductive status of prey

When examining the influence of reproductive status on likelihood of being eaten, it is difficult to separate out any influence that gender and reproductive status may have over body size, as males are larger than females. Mating pairs are typically larger than other individuals, because larger males have more success when competing for mates. No differences were seen with respect to reproductive status. Originally I thought that mate-guarding and brooding may make escape from predators more difficult, and therefore these individuals may be easier to catch (Isaksson, 1999; Rowe, 1994). This was not seen, possibly because these individuals display lower levels of activity than non-mating males and females (Chapters 2 and 3), and therefore are less prone to detection. In the presence of shelter, brooding females are less likely to encounter fish predators because of their increased use of shelter and decreased activity levels. The results could have been influenced by the tendency of some individuals (brooding females in particular) to shelter in the corners of the tank. This made it impossible for ruffe to catch these individuals. *A. aquaticus* were most commonly present along the edges of the tank, next to the glass. This made foraging attempts for ruffe more difficult, and probably also reduced the frequency by which these individuals were detected.

Prey species

Prey species as well as prey density can influence both foraging behaviour and efficiency of predators. When juvenile perch (*Perca fluviatilis*) are presented with two different prey species separately, *Daphnia magna* and *Chaoborous flavicans* larvae they forage more efficiently than when both prey species are combined

(Engelmayer, 1992). This mixing of prey species caused and increase in the time taken by perch to search and handle prey. Engelmayer suggests that this decrease in foraging efficiency is due to the use of different foraging techniques specific to each prey species, and that individuals may either have difficulty in choosing the appropriate technique to employ, or that switching between both techniques may reduce foraging efficiency. This study also highlighted the influence that prey density has on foraging efficiency, with prey search and handling time increasing when prey were present in larger numbers, but the relative composition of species was even.

Prey density

Density of prey may influence any prey selectivity displayed by predators. If prey are in low densities, fish would have to spend more time searching and therefore are less likely to be as selective than when prey are in higher densities. Factors such as these may alter the appearance of the flow diagram, as in lower prey densities there may be an increase in time spent foraging and the intensity of activities associated with hunting, such as pursuit of prey. Because of this, the predator avoidance strategies displayed by *A. aquaticus* may become less effective, as predators spend more time searching for 'frozen' individuals. Relative hunger of predators may also influence searching, with hungry predators becoming more persistent in the pursuit of prey (Wootton, 1990; Beukema, 1968). When fish were observed foraging in the presence of shelters, fewer prey were available as most individuals were present in the shelters. Under these conditions the prey densities available to ruffe were lower, and ruffe were seen to spend more time searching. The fish studied was also seen to adopt a sit and wait method of predation, waiting next to the shelters for individuals that strayed out

of shelter. Under these conditions individuals leaving shelters were very rapidly caught.

Predator-prey interactions

Behaviour of ruffe foraging on A. aquaticus

The observations made during this experiment, suggest that ruffe utilise both visual and mechanosensory cues when foraging, but that each method may be appropriate under different conditions. It is also possible that chemosensory cues may be important for foraging ruffe, and could possibly act as a trigger to begin foraging once the chemical cues from prey are detected (Atema, 1980). The detection of prey movements appears to be the major method of prey location for ruffe, whether from a visual or mechanosensory basis. This suggests that the best form of predator avoidance for *A. aquaticus* in the absence of shelter is to remain inactive. Reductions in activity are associated with predation risk in a wide variety of invertebrate species (Holomuzki and Short, 1988; McPeek, 1990).

Behaviour of A. aquaticus in the presence of ruffe

It was clear that *A. aquaticus* could detect the presence of the ruffe, rapidly reacting by sheltering in the corners and at the sides of the tank, although some individuals exposed themselves to risk by continuing to be active in central areas of the tank. Previous studies on *A. aquaticus* behaviour (see Chapters 2 and 3) have highlighted the variance in behavioural decisions within groups of *A. aquaticus*. Brooding

females have great differences in behaviour, due to brooding stage influencing behavioural decisions (Chapter 2).

On the few occasions I ran this experiment using shelters (the same as those used in chapters 2 and 3), most of the *A. aquaticus* rapidly took refuge in them. These experiments demonstrated the effectiveness of such shelters in reducing predation risk, as the fish were unable to catch prey present on the shelters. It is possible that ruffe were aware of the presence of *A. aquaticus* in shelters, because they took interest in the shelters, and often waited motionless next to them attacking prey that ventured outside. On the rare occasions that *A. aquaticus* did leave the safety of shelters, they were always eaten.

In the wild, natural substrates and benthic debris along with macrophytes would provide many natural shelters for *A. aquaticus* to hide in, and the foraging behaviour of ruffe is probably very different under these natural conditions than in these experiments. *A. aquaticus* tend to be found in dense aggregations in the wild, although most individuals would be present in more sheltered areas where predators would have difficulty reaching them. Under these circumstances ruffe would have to spend much more time seeking out prey, and as a consequence may be more persistent in the pursuit of prey than seen in these experiments.

Influence of habitat complexity on predator-prey interactions

Several studies highlight the importance that habitat complexity plays in influencing both foraging behaviour and efficiency, these factors have been well studied, especially in relation to the effect they have on inter-specific competition.

When the effect of increasing habitat complexity on survival in *A. aquaticus* under ruffe predation and *Corophium volutator* (a brackish water amphipod) in the presence perch (*Perca fluviatilis*) was studied, it was found that survival of both prey species increased with increasing habitat complexity (Mattila, 1992). Survival of *A. aquaticus* in the presence of ruffe did increase with greater habitat complexity, as the tank modifications provided effective shelter for *A. aquaticus*. Mattila also found that the ruffe fed selectively on larger individuals (with individuals larger than 10.5mm missing from the ruffe tanks), and linked this to the tendency of large individuals to be easier to locate. When relating this to the behaviour of different genders of *A. aquaticus*, it is possible that these larger individuals are males searching for mates, and therefore are out of shelter more often than other individuals, increasing the chance of being eaten. The study also highlights that the effectiveness of shelters can be dependent on the surrounding environment, where one large complex patch in a relatively barren area conveys more shelter than several small patches. This is attributed to the comparative ease with which larger shelters can be found along with the larger numbers of prey that can use bigger shelters. This is especially true in *A. aquaticus*, which are found in large aggregations amongst macrophytes.

Conclusions

This study has demonstrated that the antipredator behaviour of *A. aquaticus* can prove quite effective at avoiding predation from fish predators such as ruffe. However individuals who do not use these tactics or that are unaware of predation risk are quickly located and eaten by these well adapted fish predators.

Further areas for research

An interesting follow on study from this would be to vary the density of prey items to examine whether ruffe employ different foraging strategies at different prey densities. It would also be interesting to recreate a more natural substrate to investigate whether ruffe are able to forage among benthic substrata for prey.

Chapter 6

The incidence of macroparasites in the introduced population of ruffe (*Gymnocephalus cernuus*) in Loch Lomond

6.1 Introduction

Ruffe were first discovered in Loch Lomond, in 1982 (Maitland *et al.*, 1983). Since then their population has rapidly expanded, until they are now one of the most commonly found species of fish in the loch (Adams, 1994). It is thought that ruffe were introduced by pike anglers, using ruffe collected from sites in England as live bait. Ruffe are known to be successful invaders, from other studies in Europe, (Lake Mildevatn, Norway) (Kålås, 1995) and North America, (Lake Superior) (Pratt *et al.*, 1992). In Lake Superior where ruffe were first discovered in 1987, population expansion has also been very rapid.

Major ecological perturbations, such as invasion by non-indigenous species, have possible effects on the food web within the loch. Since the introduction of ruffe to Loch Lomond, pike (*Esox lucius*) have switched from powan (*Coregonus lavaretus*) being their dominant prey species, to ruffe (Adams, 1991). Ruffe are also influencing the powan population by feeding on their eggs, which are an important component of the ruffe diet during winter (Adams and Tippett, 1991). Ruffe also commonly feature in the diets of piscivorous birds such as the cormorant (*Phalacrocorax carbo*) and heron (*Ardea cinerea*) in Loch Lomond (Adams, 1994). Full understanding of foraging ecology of ruffe is therefore necessary as part of this study.

It was important to study the parasite status of the ruffe population in Loch Lomond, in order to relate this to the foraging behaviour and dietary preferences seen in this species.

Following previous unpublished data on the occurrence of the parasitic worm *Acanthocephalus lucii* in ruffe in Loch Lomond (D.W.T Crompton, pers. comm.), I was interested in surveying the ruffe population for this. This was parasite was particularly relevant to our study, because the intermediate host is *Asellus aquaticus*. Because of the large number of *A. aquaticus* that feature in the diet of ruffe in Loch Lomond (Adams and Maitland, 1998), I was interested in finding how this related to parasite distribution. *A. aquaticus* become infected when ingesting eggs of *A. lucii*, the larvae develops into an infective cystacanth. When the infected isopod is eaten by a fish host, such as ruffe the cystacanth will develop into an adult worm (Hoffman, 1967). In the event, we found neither ruffe nor *A. aquaticus* infected with *A. lucii*, but a survey of macroparasites in the Loch Lomond ruffe population was still undertaken.

Aims

- To investigate the structure of the ruffe population in Loch Lomond, with reference to size and gender, using a new technique for ageing.

- To examine the range of body condition and gonadal status in ruffe from the Loch Lomond population.

- To screen fish for eye and gut parasites, and relate infection to age, morphological status and foraging ecology of ruffe.

6.2 Materials and Method

Collection of ruffe

Data were collected from ruffe caught from Loch Lomond during June and July 1996. Fish were collected using fyke nets, set at depths of between 5 and 15m. After collection all fish were frozen, until being defrosted prior to dissection.

Data collected from dissections

A total of 234 fish were dissected, and a range of morphological and parasitic data were collected from them. This included standard body length (cm), total wet body weight (g), gender (based on examination of gonad structure, fish with undifferentiated gonads being classified as immature), total wet gonad weight (g), and the prevalence of eye flukes (*Diplostomum* spp.) and intestinal worms (nematodes, see below).

In addition for a subset of 150 individuals, the entire dorsal fin (including the base) was removed at the time of dissection and stored in 70 percent alcohol, for ageing at a later date (Rein and Beamesderfer, 1994).

Protocol for ageing using dorsal fin rays

Fin ray preparation

Fin rays were removed from the 70 percent alcohol, and cleaned, removing all epidermal tissue. These were then soaked in a solution of 10 percent Hydrochloric acid for approximately 10 minutes (until they were flexible). Treated fin rays were

rinsed thoroughly in distilled water and were then stored in individual vials containing distilled water until needed for sectioning.

Sectioning of fin rays

Fin rays were sectioned using a freezing microtome, (Bright starlet 2212 cryostat) kept at a constant temperature of –200°C, with the quick freeze bar maintained at -250°C. The distal end of the rays were removed prior to setting. Fin rays were frozen in Cryo-m-bed, with the proximal end uppermost. The proximal end of the ray was cut into sections 20μ thick. Good sections (that were complete and had not folded over) were removed from the cryostat by pressing a cover slip onto the section, these were then examined under a compound microscope to ensure the section is intact. All intact sections were soaked in Mayer's Haematoxylin stain for a minimum of 30 minutes, prior to examination. Under a magnification of X40, the number of growth rings in the section were counted, this gave the age of each fish.

Collection and identification of fish parasites

Parasite data was collected on eye flukes by removing both eyes; these were then either dissected straight away, or preserved in 70 percent alcohol, for subsequent study. Fluke counts were obtained by disrupting each eye in distilled water, pipetting the resulting suspension into a watch glass in small amounts, and examining under a binocular microscope. Counts were made of all the flukes present in each eye. Where the eyes had been stored in alcohol prior to parasite counts, the contents of the tube were also examined for flukes, as often they eye would have been perforated during removal. Total parasite counts were generated, and a scoring system was used to

record the intensity of fluke infection for each individual. Scores for fluke numbers were on an incremental scale, 0, 1 to 10, 11 to 50, 51 to 100 and over 100. Due to time constraints, classification of *Diplostomum* was to family only.

Analysis

Data on intestinal parasites were also collected. The intestines and stomach of each fish was removed after the removal of gonads, and was carefully opened with fine forceps. The intestines were then floated in distilled water, and examined for the presence of parasites. Any parasitic worms found, were stored in 70 percent alcohol, for identification at a later date (with the assistance of Prof. D.W.T Crompton).

Data were checked for normality and transformed where necessary (body weight was square-root transformed). Gonadosomatic indices (GSI) were calculated for all sexually mature fish, by expressing the wet weight of gonads (g) as a percentage of the total wet body weight (g), using the following equation, (Adams and Huntingford, 1997).

$$I_G = \frac{W_g}{W}$$

I_G = GSI, W_g = wet weight of gonads (g), W = total wet body weight (g).

Body condition was also calculated for males and females, in order to do this a length, weight regression was carried out, ($F_{1,227} = 1351$, $P < 0.0001$) and the resulting residuals were used as an indication of body condition.

The regression equation:

$$W = -12.1 + 2.63\ L$$

W= total wet body weight (g), L= standard body length (cm)

Data analysis

Morphometric measurements were compared between genders, using non-parametric T tests. Distribution of infection category by gender was examined by a Chi-squared test. The relationship between body condition and age, and between infection status and age, were examined by one-way ANOVA. Relationships between absolute intensity of *Diplostomum* infections and morphometric traits, were examined by regressions and distributions. The status of fish, with and without intestinal nematodes were examined by a Mann-Whitney test.

6.3 Results

General population biology

Within the sample 4 immature and 230 fish with recognisably differentiated gonads were sampled. The sex ratio among fish of identifiable gender was 62.2% female. Figure 6.1 shows a length against age distribution for all 131 fish in the aged sample. Fish ranged from 5 to 10 cm, most falling in the smaller size classes. They ranged in age from 0 to 6 years old, most being between 1 and 3 years old. As expected, most of the smaller fish were 0 or 1 years of age and most fish were aged 2+. However, a number of fish in the smallest size classes were 2 or 3 years old, while some fish of 9 cm were aged 0 or 1. Therefore growth rates in the population must be highly variable.

Table 6.1 shows the number and percentage of immature, male and female fish in each age class. All immature fish were 0 of 1 years old, but individuals of both sexes with recognisably differentiated gonads were present in all age classes. The majority of males are 1 year old, and the majority of females are 1 year, but also with significant numbers of 2 and 3 year olds. Overall there are more, older females in the distribution than males. A Chi-squared test comparing the distribution of ages in males and females, found that significantly more of the older fish were female, (χ^2 =12.9, d.f =1, P= 0.005).

Figure 6.1. a Overall length frequency for all ruffe coded for age.

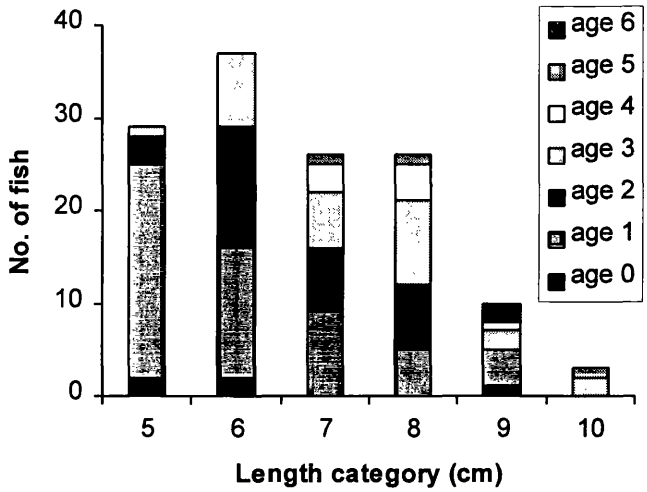


Figure 6.1. b. Overall age distribution for all ruffe.

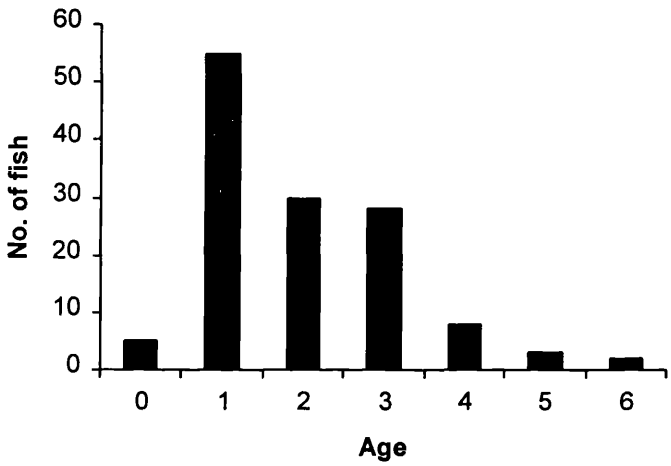


Table 6.1.

Age structure of ruffe in sample number of fish of each age in immature, female and male ruffe.

Age	Immature		Females		Males	
	No.	%	No.	%	No.	%
0	2	40.0	1	1.2	4	8.2
1	3	60.0	28	34.2	27	55.1
2	0	0	20	24.2	10	20.4
3	0	0	23	28.1	5	10.2
4	0	0	6	7.3	4	4.1
5	0	0	2	2.4	1	2.0
6	0	0	2	2.4	0	0

Table 6.2.

Mean (±SE) morphological traits for male and female ruffe, with the results of T-tests.

Morphological characteristic	Female		Male		T-test statistics
	Mean	S.E	Mean	S.E	
Body Length (cm)	7.44	0.11	6.71	0.12	$T = 4.27$, d.f = 204, $P<0.0001$
Body Weight (g)	7.55	0.32	5.51	0.28	Calculated from Sq. root of bodyweight $T = 4.77$, d.f = 223, $P<0.0001$
G.S.I	4.70	0.15	3.03	0.13	$T = 8.56$, d.f = 222, $P<0.0001$
Body Condition	0.09	0.11	-0.15	0.14	$T = 1.28$, d.f = 180, $P<0.20$

Morphological status in males and females

Table 6.2 shows the mean and standard errors for males and females, of standard body length (cm), total wet body weight (g), body condition and GSI, and also gives the T-test results for these morphological characters. Body lengths differ between the two sexes, with females being significantly longer than males and correspondingly significantly heavier. Females also had a significantly higher GSI than males. There is no relationship between GSI and body condition for either sex (for females, r=0.171, N= 142, for males R= -0.147, N= 87). Body condition did not differ between the sexes.

Age and morphological status

For both males and females, as expected, length and weight increase with age (figure 6.2). This increase is more or less steady in females, in males the rate of increase falls off from age 3, perhaps due to reproductive activity. In contrast GSI and condition do not increase with age in either gender, suggesting that most gonadal growth, occurs in younger fish (figure 6.3).

Eye fluke (*Diplostomum* sp.) infections

Figure 6.4, shows the frequency distribution of infection category in males and females in this sample. In the whole sample of fish 24.6% were uninfected. Most infected fish fell into categories 1 to 3 (1-10, 11-50, 50-100), but a significant number had very heavy infections (more than 100 flukes in total). No difference was found in the distribution of infective categories between males and females ($\chi^2 = 2.28$, d.f= 4, P= 0.69).

Figure 6.2. Length and weight in relation to age in male and female ruffe (mean ± SD). To avoid small sample sizes ages 0 + 1 have been combined in both sexes, ages 4 + 5 in males and 5 + 6 in females.

Figure 6.2.a. $F_{4,77}$ =5.41, P=0.001

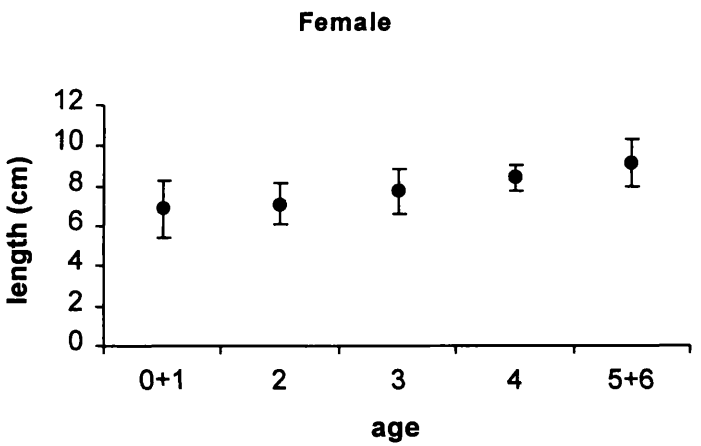


Figure 6.2.b. $F_{3,45}$ =3.37, P=0.027

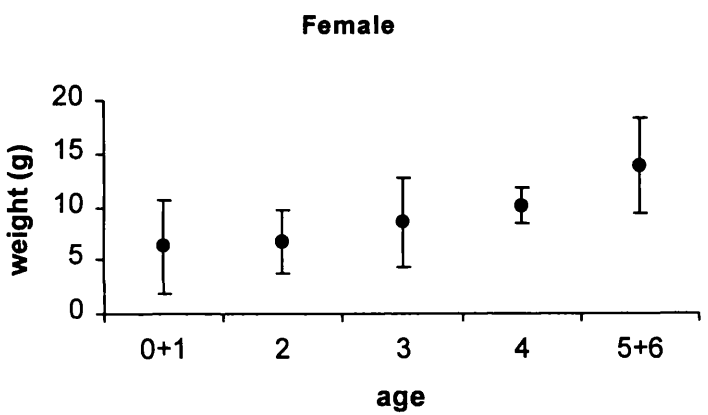


Figure 6.2.c. $F_{4,76}$ =4.61, P=0.002

Figure 6.2.d. $F_{3,45}$ =2.96, P=0.042

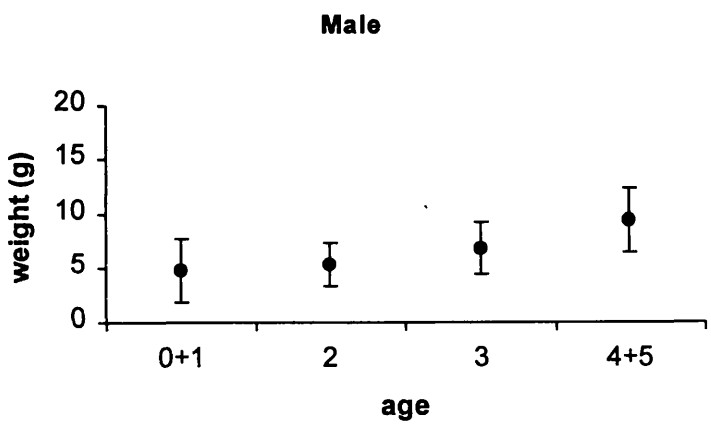


Figure 6.3. GSI and condition in relation to age in male and female ruffe (mean ± SD). To avoid small sample sizes ages 0 + 1 have been combined in both sexes, ages 4 + 5 in males and 5 + 6 in females.

Figure 6.3.a. $F_{4,75}=1.95$, P=0.11

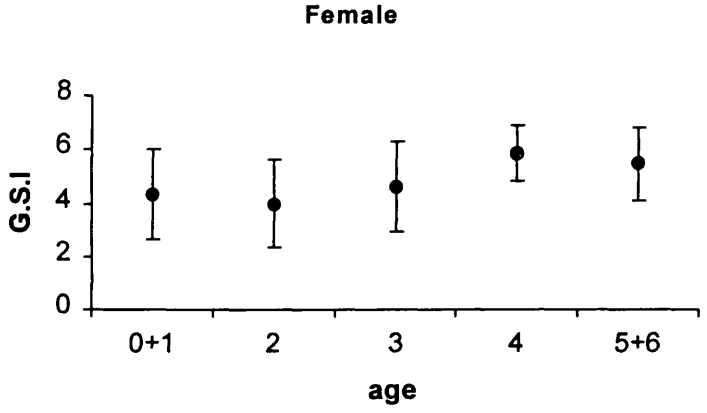


Figure 6.3.b. $F_{3,43}=1.0$, P=0.40

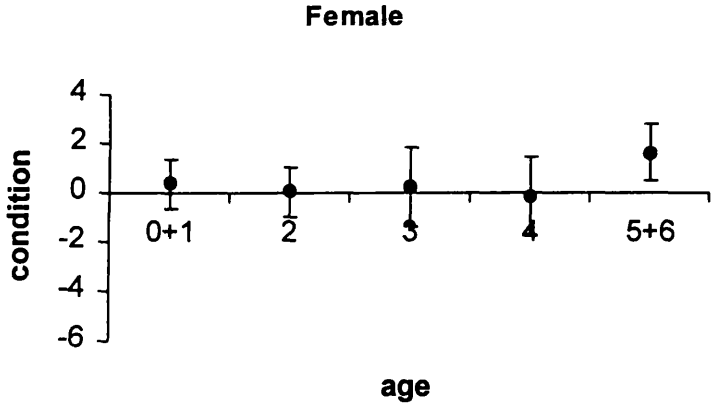


Figure 6.3.c. $F_{4,76}=1.51$, P=0.21

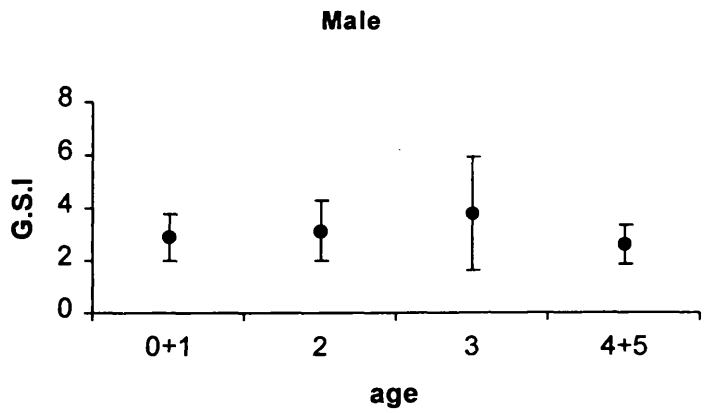


Figure 6.3.d. $F_{3,45}=2.19$, P=0.10

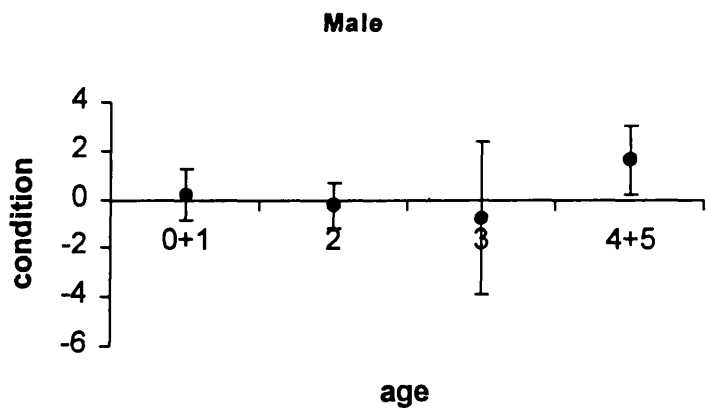


Figure 6.4.

Frequency distribution of categories of infection with *Diplostomum* spp., coded by gender for a subset of 189 individuals from the sampled population.

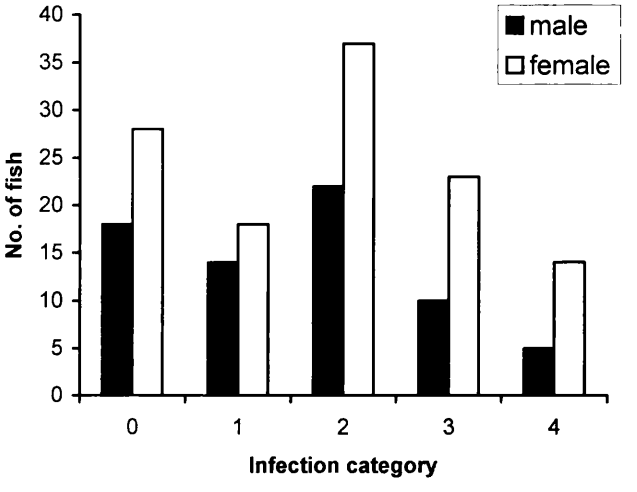


Table 6.3 shows the distribution of infection categories with age and the median category for each age. In general, lower levels of infection tend to predominate in younger fish, the result that average levels or infection increase with age, (Kruskal-Wallis, H= 25.88, d.f= 6, P<0.001). Figure 6.5 shows mean levels of 4 morphological traits in relation to infection category, together with the results of one-way analysis of variance. Length, weight and body condition all rose significantly with increased infective status. There was a marginally significant association between infective category and GSI, levels being lowest in categories 1 and 2.

Table 6.4 shows the results of regression analysis using total numbers of *Diplostomum* found in each fish (rather than infection category). Univariate regressions show a significant positive association between number of *Diplostomum* and age, length, weight, condition and GSI, (table 6.4a). Because these variables are related, a multiple regression was carried out to identify the key effect, (Table 6.4b). This showed age to be the most significant predictor of intensity of infection. The relationship between intensity and age is shown in figure 6.6.

Nematode infections

A small proportion of the fish sample (12 in the whole sample) had gut worms (Table 6.5), all of the same species of nematode, *Camallanus lacustris* (Zoega, 1776). Eleven of these had just one nematode, and one had three. Infected fish were found in age classes from 0 to 4 and ranged in length from 5.3 to 8.7cm. Thus there is no detectable trend with age and size.

In females the ratio of nematode infected to uninfected was 7:143, in males this ratio was 5:87. When analysed using a Chi-squared test no differences were found with frequency of nematode infection between the sexes (χ^2=0.07, d.f= 1, P= 0.79). Even though the number of infected fish was relatively low, fish with worms and without worms were compared. Table 6.6 shows the median lengths, weights, GSI and body condition in ruffe with and without worms, plus the results of a Mann-Whitney test. There were no significant differences between fish with and without worms, indicating that acquisition of this parasite is independent of size and condition.

Table 6.3.

Distribution of infection categories in relation to age in all ruffe.

Age	Infection category					
	0 (0)	1 (1-10)	2 (11-50)	3 (51-100)	4 (>100)	Median infection category
0	2	2	1	1	1	1.0
1	4	23	20	7	4	2.0
2	0	5	20	5	0	2.0
3	0	3	11	9	5	2.5
4	0	1	1	3	3	3.0
5+6	0	0	2	2	1	2.8

Table 6.4.

Regression analyses of absolute numbers of *Diplostomum* (D) in relation to morphological variables.

a) Univariate Regressions

Variable	**Regression Equation**	R^2	$F_{1,135}$	**P**
Age (A)	D=15.1 + 11.8	17%	28.0	<0.001
Length (L)	D=-67.5 + 14.9 L	33%	66.3	<0.001
Weight (W)	D=4.96 + 4.94W	32%	66.5	<0.001
Condition (C)	D=37.7 + 5.31C	3%	5.65	=0.02
G.S.I (G)	D=12.7 + 6.82G	10%	15.7	<0.001

b) Multiple Regression

Regression Equation	R^2	$F_{1,135}$	**P**
D=-102 + 4.91A* + 21.7L – 3.73W + 6.37C + 0.15G * T=2.13, P=0.04. Other variables NS.	33.5	13.7	<0.001

Figure 6.5. Mean ± SD of morphological traits in relation to infection categories for all fish a) length,b) weight, c) condition, d) G.S.I (1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = >100).

Figure 6.5.a. $F_{4,184}$ =10.62, P<0.001

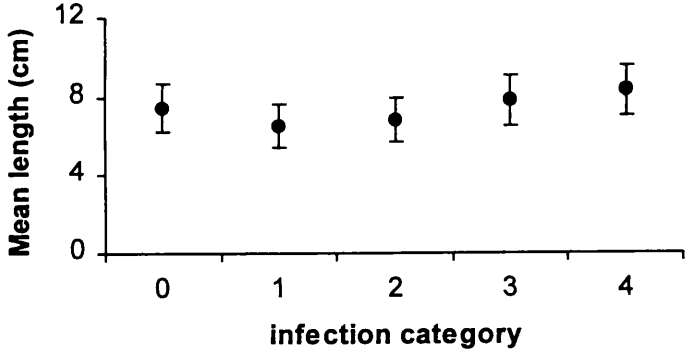


Figure 6.5.b. $F_{4,183}$=11.53, P<0.001

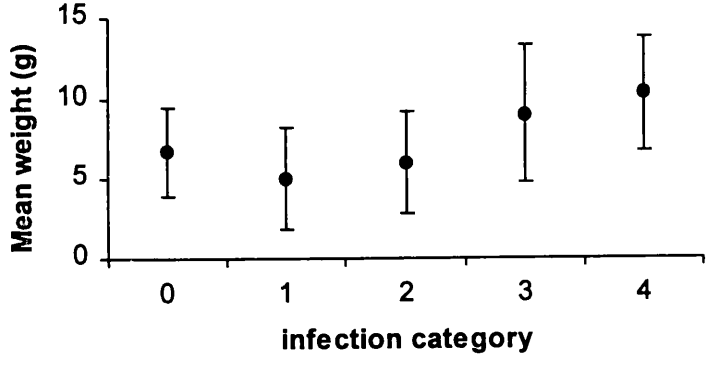


Figure 6.5.c. $F_{4,183}$=6.10, P<0.001

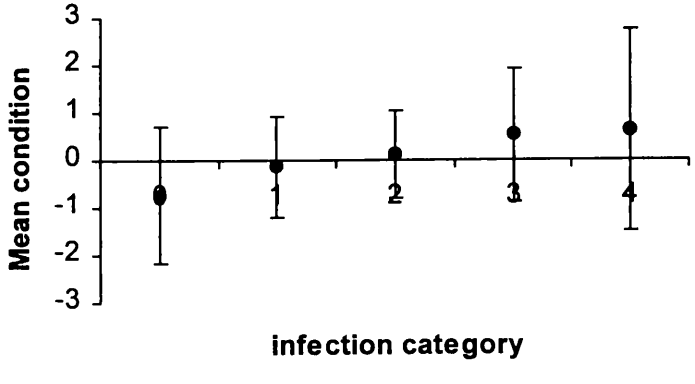


Figure 6.5.d. $F_{4,180}$=2.33, P=0.057

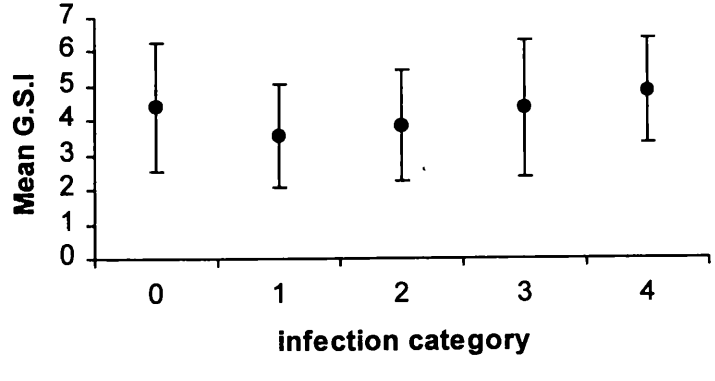


Figure 6.6. Absolute number of *Diplostomum* larvae in relation to age for all fish.

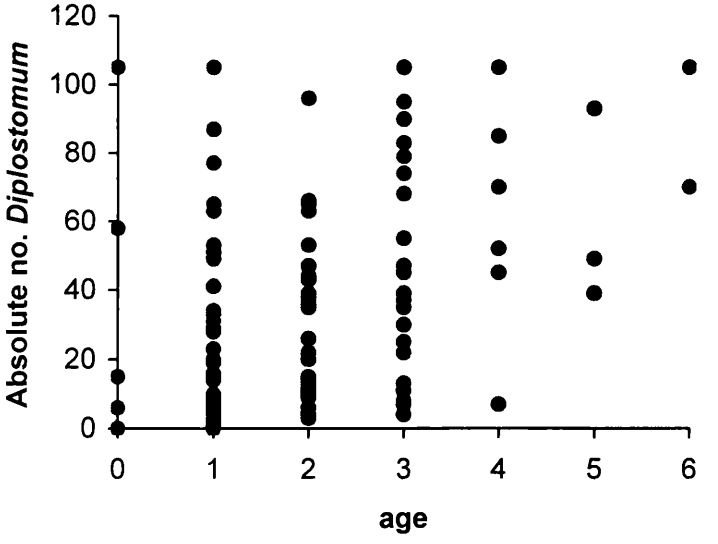


Table 6.5a

Morphological data for male ruffe with gut nematodes (*Camallanus lacustris*) (Zoega, 1776)

Fish No.	Standard Length (cm)	Body Weight (g)	Gonad Weight (g)	GSI	Age	total no. eye flukes	no. worms
23	7.8	7.16	0.2	2.79	n/a	>100	1
47	6.5	5.06	0.14	2.77	n/a	<100	1
193	5.9	2.6	0.05	1.92	n/a	<10	1
195	7.3	7.4	0.18	2.43	0	<50	1
210	7	n/a	0.17	n/a	2	<100	1

Table 6.5b

Morphological data for female ruffe with gut nematodes (*Camallanus lacustris*) (Zoega, 1776)

Fish No.	Standard Length (cm)	Body Weight (g)	Gonad Weight (g)	GSI	Age	total no. eye flukes	no. worms
91	7.1	6.61	0.42	6.35	n/a	<100	1
115	6.9	5.8	0.28	4.83	4	<50	1
130	7.5	8	0.46	5.75	2	<100	1
158	8.7	11.8	0.54	4.58	3	<50	1
161	9	11.9	0.99	8.32	n/a	<50	3
174	8.2	9.9	0.39	3.94	1	<50	1
203	5.3	2.8	0.05	1.79	1	<50	1

Table 6.6.

Median values for morphological variables for ruffe with and without nematode worms, with the results of Mann Whitney tests.

Median:	With worms (12)	Without worms (230)	W	P
Length	7.2	7.1	27852	0.69
Weight	7.2	6.0	27475	0.60
Condition	17.92	16.93	27417	0.43
G.S.I	3.94	4.0	26907	0.96

6.4 Discussion

Age, size and reproductive status of ruffe

In this sample of ruffe, fish ranged in age from 0 to 6 years old, and in size from 5 to 10cm. Maitland and Campbell (1992), observed that ruffe generally live between 4 to 6 years, which explains why we saw relatively few fish older than 3 years, and no fish more than 6 years old.

Mean GSI for males was 3.03 and for females was 4.7. These are quite high figures for males. GSI was unrelated to body condition in our sample of fish, which suggests that gonad development does not occur at the expense of energy reserves.

GSI can fluctuate greatly, due to the annual reproductive cycle. The GSI observed here for females, are relatively small in comparison to other fish species. This could be because of the stage in the annual cycle. These fish were caught in June, but spawn between March and May (Maitland and Campbell, 1992) and therefore their ovary mass may have been depleted following spawning. The ovaries of female salmonids can comprise 20 to 30 percent of the total body weight just prior to spawning (Wootton, 1990). In other species, such as the cichlid (*Orechromis leucostictus*), the ovaries form just 3 percent of body weight.

The GSI observed in male ruffe was relatively high when compared to some other species. In sticklebacks, prior to spawning male testes comprise under 2 percent of body weight, whereas females ovaries form 20 percent of body weight at the same

point (Wootten, 1990). GSI did not increase with age, which suggests that active reproductive development occurs in young fish. This is supported by previous studies that have suggested that male ruffe become sexually mature 1 year old with females maturing later at 2 years old (Varley, 1967). The tendency for ruffe to mature rapidly and have comparatively large gonads, may go someway to explaining why the population expansion of ruffe in Loch Lomond was so rapid. However, a study on the reproductive characteristics of female ruffe in Lake Superior, found that GSI was not a reliable predictor of spawning potential, when compared with histological examinations of ovaries (Leino and McCormick, 1997). In that study some young fish had high GSI values (for example a one year old had a GSI of 9.7), but had immature oocytes. Most age 1 and 2 fish did not spawn, instead they reabsorbed their oocytes, with only approximately 20 percent of 2 year olds spawning. A relatively long spawning period was observed, from late April to mid June (in the year 1994), with larger females producing more than one clutch during this time.

Parasites

Fish were in good condition, and when screened for macroparasites, generally few were found, especially in the gut.

Nematodes

Parasitic nematodes were very rare in our sample of ruffe. It is possible that this could be related to the lifecycle of *Camallanus lacustris*, which makes it less likely to be transmitted to ruffe. *Camallanus lacustris* (Nematoda) is common amongst a variety of freshwater fish, including ruffe and perch (*Perca fluviatilis*) (Kennedy, 1974;

Moravec, 1994) which are the definitive hosts of this parasite. The life cycle of *C. lacustris* includes a larval stage within the intermediate host, a copepod belonging to the genus *Cyclops* (Hoffman, 1967). Copepods ingest the larvae of this parasite, which then develops in the host body cavity. Only copepods with third stage larvae are infective to fish, which become infected from eating these copepods (Moravec, 1994). Within Loch Lomond copepods comprise a very small percentage of the ruffe diet. In the summer they make up 1.7 percent of the diet, and in the winter this rises to 3.5 percent (in the mid-basin) (Adams and Maitland, 1998). Because they are rare in the diet of ruffe, it is unlikely that this parasite would be widespread in the Loch Lomond ruffe population.

Diplostomum

There was a high incidence of *Diplostomum* infection in eyes of the fish sampled. *Diplostomum spathaecum* has been previously found in ruffe in Britain (Kennedy, 1974). No gender differences in occurrence or intensity of infection were observed, and no adverse effects of infection on body condition or GSI were seen. There was a strong positive association with age and a weaker association with size. This implies that there is a non-selective accumulation of parasites through prolonged exposure. High incidence of *Diplostomum* infection has been previously observed in other benthic species, including the arctic charr (*Salvelinus alpinus*) (Dorucu *et al.*, 1995; Dorucu, 1996).

Fish become infected when the free-swimming cercariae of this parasite burrow through the skin and migrate to the eye, where fish can accumulate large numbers of

metacercariae (Crowden and Broom, 1980). It is thought that benthic species have a higher exposure to the cercariae of *Diplostomum*, partly due to the abundance of the intermediate snail host in the benthos (from which the cercariae are released); but also because deeper water is calmer, which aids swimming and therefore host location in this species, (Davis and Huffman, 1977). It is therefore possible to attribute the high incidence of *Diplostomum* infection in ruffe to their benthic habitat, which increases their exposure to this parasite.

Diplostomum infections are linked with impaired vision in other fish species. A study on the feeding behaviour of the visual predator dace (*Leuciscus leuciscus*) on the amphipod *Gammarus pulex*, associated heavy *Diplostomum spathaceum* infections with a number of altered behaviours (Crowden and Broom, 1980). As the intensity of infection increased, there was a reduction in successful feeding attempts, and an increase in the time spent foraging (at the surface). The distance by which fish reacted to prey also decreased with increasing parasite burden. Although *D. spathaceum* infection could not be associated with a decrease in body condition in dace, it does bring about behavioural changes which reduce foraging efficiency and increase predation risk from piscivorous birds. A more recent study in sticklebacks (*Gasterosteus aculatus*) found that even relatively low *D. spathaceum* eye infections (of up to 9 metacercariae), significantly reduced the preference shown towards the prey species *Asellus aquaticus*, when compared to less heavily infected eyes (Owen *et al.*, 1993). They also found that infected fish had a significantly shorter reaction distance when feeding on *Daphnia* sp.

The number of *Diplostomum* present in fish eyes accumulates over time, and therefore it was surprising that the older fish (above four years) did not appear to have higher absolute numbers of flukes than younger fish (figure 6.6). This may be due to the fact that *Diplostomum* infections reduce the ability of fish to avoid predation, and to forage effectively. Fish with particularly heavy infections, may be removed from the population as a result of increased predation. The reduction in visual acuity resulting from heavy infections may directly increase predation risk, as fish may not see oncoming predators. An additional factor may be caused by reduced foraging efficiency (as a result of reduced vision), resulting in fish having to spend more time foraging in potentially dangerous areas, putting individuals with heavy parasitic burdens at a greater risk of being detected by predators.

When considering the effect that *Diplostomum* infections have on visually foraging predators, perhaps the ability of ruffe to use non-visual cues when foraging (reviewed in chapter 4, Disler and Smirnov, 1977; Janssen, 1997), explains the lack of negative effect in condition in even relatively heavily parasitised fish.

Chapter 7

General Discussion

7.1 General Aims of the project

The overall aim of this project was to learn more about the foraging behaviour of ruffe, and to relate this to predator avoidance behaviour and the trade-offs involved in a major prey species of ruffe. In this chapter I will discuss the findings of my research, and how this relates to existing studies, taking the topics in each chapter in turn.

7.2 Anti-predator adaptations in *A. aquaticus*

7.2.1 *Shelter use and foraging*

The study on shelter seeking and foraging in *A. aquaticus* (described in Chapter 2) demonstrated that reproduction influences the behavioural trade-offs between foraging and shelter. Costs of reproduction to males are a result of the energetic demands associated with guarding, combined with the avoidance of competing males. This results in a decrease in activity, and a higher proportion of time being spent in shelter. This demonstrates that mate-guarding in males influences the trade-off of feeding against shelter use. The behaviour of females was also influenced by the brooding cycle. A variety of factors may influence the costs and benefits of choosing to feed, as opposed to taking shelter. This study highlights the importance of shelter during early and middle stages of brooding, but I suggest that females balance this against the need for their young to be released in to profitable habitats, and the necessity of replenishing lost food reserves immediately after the cessation of brooding.

7.2.2 *The behavioural responses of A. aquaticus to predator cues*

Existing studies demonstrate the importance of chemical cues in detecting the presence of predators in several aquatic invertebrates. In this study I found that *A. aqauticus* modified their behaviour in the presence of chemical cues, reducing activity and an increasing their use of shelter. This trend was also seen in control runs and therefore temporal effects appear to exert strong influences on behaviour in *A. aquaticus*, I was therefore unable to ascertain whether the presence of cues from fish mediated anti-predator responses in this prey species.

7.2.3 *Antipredator adaptations and behavioural trade-offs in Asellus aquaticus*

Previous studies have largely neglected the role of brooding in female isopods, as a costly life history adaptation. I was interested in finding out whether the behaviour of different reproductive categories was different, and whether any differences in behaviour could be related to a change in predation risk induced by reproductive status (Chapter 2). This investigation revealed several interesting aspects of the behaviour of *A. aquaticus* in relation to their reproductive status.

Males

I found that single males had higher activity levels than the other reproductive categories tested. This is in keeping with existing studies on other isopod species, and suggests that males during the breeding season spend a large proportion of their time seeking out mates.

Behaviour of males was modified when guarding females. This can be explained in terms of a number of costs that are associated with guarding in this species. Firstly, the act of carrying a female can be energetically costly, and has been shown to reduce glycogen reserves in guarding males when food is absent (Sparkes *et al.*, 1996). In the presence of food, mate-guarding males displayed elevated foraging rates, presumably to reduce the energetic costs of guarding.

By hiding in refuges during mate-guarding, males are not only reducing energetic costs, but are also reducing the chance of take over attempts from competing males. These take over attempts have several associated costs; mainly the male risks losing his mate, but also physical damage to both the male and female can occur as a result of these struggles, in addition to elevating energetic demands.

The mobility of males and females has been shown to be reduced during mate-guarding (Adams *et al.*, 1985; Hatcher and Dunn, 1997), and therefore the risk of predation may also increase. This supports the view that reduced activity and increased shelter use in mate-guarding males, demonstrated in Chapter 2, is an adaptation to avoiding predation.

The costs associated with mate-guarding for males support the results found in Chapter 2, where pairs of *A. aquaticus* in precopula used shelter more than non-mating males and females, and displayed lower levels of activity. Therefore males in my experiments were trading-off foraging, during an energetically expensive stage in their life history, in order to reduce the costs associated with guarding females.

Females

One of the most interesting findings of the work described in this thesis, were the behavioural changes observed during brooding in females (Chapter 2). I found that brooding females had both lower activity levels and used shelter more than non-breeding males and females. This confirmed that brooding was influencing behavioural decisions in females. However the most interesting results I found were when individual behaviour was studied during the brooding cycle. Females in the early stages of brooding showed a weak trend of feeding more than those in the middle stages of brooding, although this was not significant (Chapter 2). Other isopod species have shown that prior to brooding, females display elevated feeding levels, and it has been suggested that they do this to accumulate the reserves required for brooding, when they do not appear to feed. In my study, females in the middle stages of brooding spent significantly longer in shelter than females in the final stages. The behaviour of females nearing the end and immediately after brooding was very different from this. Individuals were observed outside of shelter and near food more often in the days immediately prior to brood release, where those immediately after brood release fed significantly more. These results suggest that a trade-off exists between seeking shelter for safety during brooding, and maximising habitat profitability for newly emerging young. The increased feeding observed at the cessation of brooding, also confirms that during brooding, females reduce their food intake in preference for shelter (during daylight hours), and that in order to reduce the fitness costs associated with these behavioural decisions, food intake must be maximised once brooding ends.

Brooding may influence behaviour in this way for different reasons. Although the costs to females of carrying a brood has not been studied, it is possible to speculate as to why shelter use and reducing activity may be important for females. Mate-guarding in males has been shown to incur energetic costs, it could be true that the burden of carrying a brood increases energetic demands in females. This has not been studied, but could influence the behavioural decisions of females. Lyndon (1996) noted that brooding females have difficulty 'righting' themselves when they are knocked over. This suggests that the mobility of females is reduced when carrying broods. This could cause difficulties when evading predators, but also could cause problems when moving in currents (Adams *et al.*, 1985). Therefore using shelter could reduce the problems associated with mobility when carrying broods, in addition to decreasing the risk of predation. Shelter use has been shown in previous studies on isopods to be an effective strategy against fish predators (Holomuzki and Short, 1988).

Shelter use was shown to be an effective predator avoidance strategy when behaviour was studied in the presence of ruffe (Chapter 5). Foraging ruffe were unable to attack individuals hidden amongst the artificial shelters, confirming that the behavioural decisions made by both brooding females and mate-guarding pairs increase their chances of survival from fish predators.

Direct observations on the behaviour of brooding females were not made in this study, merely their activity within tanks. A recent study on brood care in the amphipod

Crangonyx has shown that females display active brood care throughout the development of their young (Dick *et al.*, 1998). The intensity of this brood care varies corresponding to developmental stage. This supports the suggestion that females adjust their behaviour in the final days of brooding, in order to release their young in a profitable habitat. In the present study females were seen in feeding area (but were not observed feeding) more towards the end than in the middle stages of brooding, so it may be that similar maternal responses are shown by *A. aquaticus*. Shelter use in brooding females may be more than just an adaptation to elevated predation risk and energetic demands. If so, the possibility that females could be caring for their broods whilst in shelter, may in some way explain why females incur the fitness costs associated with lost feeding opportunities, as a result of remaining in shelter during the brooding period.

7.2.4 *A. aquaticus behaviour related to predation risk*

The high activity levels of males (Chapter 2), most likely a result of mate-seeking, would appear to put them at a higher risk of predation. This is supported by my studies on the foraging behaviour of ruffe, because ruffe prey detection is based largely on prey movements (Chapter 5). However, when this was tested I found that ruffe did not selectively feed on any one particular gender. It is therefore possible that although males may appear to be at a higher risk of predation due to their mate seeking behaviour, that they may adapt their behaviour when under predation risk. This suggestion that males adapt their behaviour when under threat of predation was partly upheld by experiments carried out in Chapter 3. Males were found to significantly decrease their activity, and spend longer in shelter in the presence of

chemical cues from potential predatory fish. Although this change in behaviour was not sufficiently different from control studies to confirm that the presence of cues alone mediated this behavioural response, the change in behaviour observed in males was stronger in the presence of cues than when they were absent. It is also possible that single males are better at escaping from predation than other reproductive categories of *A. aquaticus*. From observations made during ruffe foraging experiments (Chapter 5) fleeing did not prove a particularly effective escape response in dim light. However in a small number of cases, larger males were able to escape ruffe by fleeing. This was not an option for smaller individuals, which were eaten in one bite. Larger individuals were handled for longer, therefore giving them more time to escape; on a very small number of occasions large males successfully escaped by being spat out by the fish. Although I only studied behavioural responses of *A. aquaticus* to fish predators in this thesis, there are many invertebrate predators of *A. aquaticus*. Certain amphipods are known predators of *A. aquaticus* (Bengtsson, 1982), and it is possible that the larger body size of males, is effective when evading predation from small predators. This phenomenon was seen in the isopod *Lireceus fontinalis*, where individuals were able to avoid predation by gape-limited salamander larvae by reaching larger sizes (Sparkes *et al.*, 1996). The predation risk of different genders of *A. aquaticus* may be dependant on body size, and may not be an important factor when considering predatory threat from large predators such as large fish. However it may be an important factor in determining behavioural responses in relation to other smaller predators.

7.2.5 *The influence of chemical cues from potential predators on A. aquaticus behaviour*

The behaviour of brooding females in my study in chapter 2 may go some way to explaining the fact that they did not react to the presence of chemical cues (chapter 3). Brooding females were already spending much of their time in shelter, and therefore I was less likely to see significant effects, such as increased shelter use than with males. A surprising result was the amount of time that non-brooding females spent in shelter; this did not correlate to the behaviour seen in my study in chapter 2. The design of both these experiments (chapters 2 and 3), was intentionally very similar, in order to enable direct comparisons. I was surprised that temporal differences between days produced such a strong behavioural effect in the control trials. Possibly this reflected generally low levels of activity by individuals during daylight, and that activity and feeding was higher overnight. Previous studies have demonstrated the influence of chemical cues from predators on invertebrate behaviour is greater at night, presumably because visual cues can be used during the day to confirm that no predators are present.

Although I could not prove that *A. aquaticus* respond to chemical cues from fish predators, I feel that they must have the ability to detect these cues. Previous studies on amphipods and isopods (Bengtsson, 1982; Holomuzki and Short, 1988; Wooster, 1998) have shown that the presence of chemical cues from predators, elicits avoidance behaviour.

If I was to repeat this experiment (chapter 3), there are numerous improvements that could be made to this set of experiments. I think that the main problems my design had was a result of limited space, which reduced the number of control trials I carried out, and prevented me from running control and experimental trials simultaneously. Two additional tanks would have allowed me to run good controls and also to have a tank of predatory fish, such as perch that I could feed with *A. aquaticus*. The experimental design was overly complex, as I was attempting to examine individual behavioural changes in the presence of chemical cues. To examine possible effects of cues on group behaviour, it was not necessary to use pumps to fill the tanks. Instead static tanks containing different individuals run at the same time could have been compared for activity and shelter use in the presence and absence of predatory cues.

Another source of error could have been the use of water from Loch Lomond for the control runs. This would have contained fish cues, and it is possible that the accumulation of these cues could have influenced daily activity within the control tanks.

I realise that the experiment described in chapter 3 is relatively weak, and with the benefit of more time, I would have attempted to repeat these experiments in the variety of ways described above (and in chapter 3). I do feel that this is still an important area of research, especially in the implications for predator-prey interactions between *A. aquaticus* and ruffe, where non-visual cues appear to be important.

7.3 Ruffe as foragers

7.3.1 *Ruffe foraging in the absence of visual cues for concealed prey*

The foraging behaviour of ruffe was examined in the dark, when foraging for concealed prey within an experimental arena. This study highlighted the difficulties of observing foraging behaviour of fish in the dark, and demonstrated that ruffe can use non-visual cues to locate concealed prey.

From studying the foraging behaviour of ruffe foraging in the dark for buried bloodworm, with the aid of infrared light and infrared sensitive video equipment, I found that ruffe can forage effectively in the absence of visual cues (chapter 4). All fish visited pots containing food more often than those without food, and most of the fish visited food pots significantly more than would be expected if pot visits were random. There appear to be two different foraging strategies that are employed by foraging ruffe under these conditions. Some of the fish discriminate between pots with food in and those without, visiting each pot for a relatively short time, but learning throughout the experimental period which pots contain food. The other method employed by the rest of the fish, is more random in nature, with fish being more random in their choice of pots to visit. However, once there they spend much longer foraging in pots with food, maximising their energy gain for each foraging visit made. These fish do not display learning as the other fish, and therefore are definitely showing a different type of foraging pattern.

Fish that use the less random method of foraging, must be utilising cues in order to decide which pots to visit. However they do not spend very long visiting each pot, but instead visit many pots for a shorter duration. This method appears to promote learning, as although fish spend less time at each pot, they may be gaining more spatial information as the forage between pots more often. In these fish it is clear that the use of non-visual cues is important in foraging decisions, but that foraging becomes more successful after learning has occurred. It is possible that by using this strategy these fish are treating the arena as one foraging patch, which contains some profitable and other non-profitable areas.

It is possible that fish that foraged within pots for less time were actually better at finding and catching prey, and therefore they did not need to spend a long time foraging at each pot. This may explain why fish 4 switched foraging modes, possibly it took them longer to learn how to forage from pots, and they had become more efficient at extracting prey from pots after the first 2 experimental runs.

Fish that approach foraging decisions in a more random way, do still visit pots containing food more often than non-food pots, and therefore must be using some form of cue. However they do not display learning as the experiment progresses, and instead visit fewer pots for longer. This is a more efficient way of foraging, providing that by spending longer at pots they increase the amount of food taken with each foraging visit. These fish may be better at picking up cues when in close proximity to prey, rather than using spatial memory.

The two different modes of foraging can be discussed in relation to marginal value theorem. Fish that spend less time at pots may have a higher threshold than those foraging longer at pots, and it is possible that this is influenced by factors such as hunger. Fish 4 shows the interesting pattern of switching between these foraging modes, as in the first 2 trials with this fish, it forages in a more random way, whereas in the last trial it appears to display learning. The ability of a fish to display both modes of foraging suggesting that fish adapt their foraging behaviour, either through experience or possibly due to hunger or energy costs. Hungry, less energetic fish may prefer to visit fewer pots, foraging longer when food is found (maximising food intake for food searching time), this fits with the marginal value theorem, where particularly hungry individuals may lower their threshold value for a patch before moving on to the next one.

Foraging bout durations showed a trend in some fish to increase as the experiment progressed. This may be expected as the amount of food within a patch decreases, the length of time taken to find the available food within pots should increase, in relation to the decrease in food abundance. However, there was no clear indication that this was occurring, and instead decisions on foraging durations may be more random.

It is clear from the results of this experiment that ruffe can forage in the absence of visual cues, but that this requires a high level of activity, and that seeking out buried prey in the dark increases the amount of effort required for foraging. When compared to the data collected during ruffe foraging under very different conditions (in chapter 5), the activity levels associated with foraging are very high.

This experiment could be greatly improved by increasing the numbers of replicates, for each fish and also studying more fish. This was a very difficult experiment to set-up, and training the fish was a lengthy process that required great attention. Due to the need for filming under infrared, I was limited to using only one tank, which is why the experiments took such a long time. In order to fully categorise and quantify the foraging behaviour of these fish, video analysis proved a limiting factor on the number of tapes which could be studied.

Following on from these experiments, there are several further areas for investigation. The foraging behaviour of ruffe could be studied on different substrate types and for different prey. Comparisons of foraging behaviour and effort could be made when foraging in muddy sediments and gravel sediments, for different species of prey. It would also be possible to investigate which non-visual senses fish were employing during foraging, by using combinations of dead prey and vibrating artificial prey.

7.3.2 *Behavioural responses of ruffe foraging on A. aquaticus and predator avoidance tactics by prey*

Behavioural interactions between ruffe and *A. aquaticus* were examined. This study highlighted the importance of shelters to prey species such as *A. aquaticus* in providing effective refuges from fish predators; and to confirm that increased shelter use by individuals is an effective anti-predator strategy.

Observations of the foraging behaviour of ruffe in dimly lit tanks demonstrated the importance of prey movement in the location and capture of prey in this fish species. This also suggests that a combination of both visual and mechanosensory cues are important when searching for prey.

Observations of the escape tactics of *A. aquaticus* showed how prey can exploit the dependence of predators on movement when detecting prey. *A. aquaticus* were able to successfully evade predation by remaining still, thus avoiding detection. Individuals that displayed flight responses almost always failed to escape predation. This study was important in relating the behaviour of both species together, in order to comment on the predator-prey relationships between them.

From my direct observations on the behaviour of ruffe foraging on *A. aquaticus*, I gained information on the factors that are important to ruffe when foraging. These experiments were conducted in dimly lit tanks, providing enough light for ruffe to utilise visual cues in foraging. The prey were not only more visible due to the lighting conditions, but were also relatively active and mobile. Under these conditions ruffe spent very little time actively foraging. Instead the majority of time was spent inactive. Ruffe appeared to forage periodically, although most foraging attempts were made when active prey were detected in close proximity. This use of prey movements in the detection of prey indicated that ruffe were using a combination of both visual and mechanosensory cues in foraging.

The low levels of activity displayed by ruffe were in stark contrast to the behaviour of ruffe foraging in the dark. It is likely that ruffe were foraging more efficiently in the light, as very little energy was used when searching for prey. The differences in these studies highlight the influence environmental conditions such as light intensity have over foraging abilities in fish.

Behaviour of *A. aquaticus* in the presence of ruffe was similar to that of isopods in previous studies (Holomuzki and Short, 1988), and also related well to the work on shelter seeking in *A. aquaticus* described in chapters 2 and 3. When shelters were present most individuals hid amongst them, ruffe were unable to hunt within shelters and therefore this proved an effective predator avoidance strategy. In experiments manipulating levels of habitat complexity when ruffe foraged on *A. aquaticus*, it was found that increasing levels of complexity increased the survival rates of prey (Mattila, 1992). The greatest protection from predators was provided by large refuges surrounded by an area of low complexity. This may be because large shelters are easier to locate, and more prey can use them. This may be especially important for prey such as *A. aquaticus* that show a tendency to aggregate (chapter 2).

The isopod *Lircues fontinalis* displayed a strategy of becoming totally inactive when within 10cm of a predatory green sunfish (Holomuzki and Short, 1988). *A. aquaticus* in this study used this method of predator avoidance, which was especially important at evading predation in the absence of refuges. This demonstrates that *A. aquaticus* are not only conscious of the presence of predatory fish, but that they are able to exploit the reliance of ruffe on reacting to movements when locating prey. A less

common escape tactic used was fleeing. This proved less effective at evading predation, but was occasionally successful, with fish either not bothering to chase an individual, preferring to pursue a less evasive individual. This behaviour has been seen in a previous study on response to predation in *A. aquaticus*. Verrell (1985) studied behavioural responses of *A. aquaticus* when under predation threat from salamanders (*Triturus vulgaris*). He found that in 50 percent of occasions, when a pair in precopula were pursued by a newt, the male released the female and ran away, resulting in the male escaping but the female being eaten. This study also found that the high levels of male activity put them at a higher risk of predation than when they were mate-guarding, as were less active. However the large size of precopula pairs made them more attractive to the salamanders. Detailed behavioural observations were not possible in this study, as visibility of the tank base was reduced by foraging ruffe, which often disturbed the substrate when attacking prey.

Prey selection

The genders and sizes of prey eaten by ruffe were studied, and no selection of *A. aquaticus* by size or gender was seen. A previous study of ruffe predation on *A. aquaticus* found that ruffe selected prey on a basis of size (Mattila, 1992). In control tanks containing no ruffe the mean body length of surviving individuals was 6.9mm, in ruffe tanks this was 6.2mm, the largest individuals (above 10.4mm in length) were also missing in the ruffe tanks. Mattila suggests that this is due to larger prey being more conspicuous and therefore at a greater risk of predation. While this is true, it is also likely that these individuals were males (due to their large size), which have been shown to display higher levels of activity than females (chapter 2; Verrell, 1985),

which would ultimately expose them to higher levels of predation. It was therefore surprising that ruffe did not selectively feed on particular size classes or genders in my experiment. It is possible that size selection was reduced due to the relatively low density of prey within the experimental tanks. This was exaggerated in the presence of shelters, and also by many individuals hiding in the corners of the experimental tanks preventing ruffe from eating them. Previous research has indicated that when prey are at low densities predators become less choosy, and that the same individuals display high prey selectivity when food is at high densities.

The results of the experiments describing *A. aquaticus* behaviour in the presence and absence of ruffe relate well to each other. The high levels of protection provided by shelters when under threat of predation from ruffe confirmed that the behavioural adaptations of brooding females and pairs in precopula of reducing activity and increasing shelter use will result in reducing risk from predators. This also highlights the risks associated with the behavioural decisions of females to leave shelter in the latter stages of brooding, suggesting that prolonged time in shelter occurs as a result of trade-offs between foraging and reducing predation risk. It also demonstrates that these trade-offs are not constant, but are balanced with the requirements of the developing brood and nutritional status.

The study on behavioural interactions between ruffe and *A. aquaticus* could be improved in a number of ways. If the experiments had been recorded on video, it would have been possible to examine specific aspects of the behaviour of both species more closely. This may have provided data on escape rates of individuals and the

number of successful and unsuccessful attacks by ruffe on *A. aquaticus* employing different escape tactics could have been quantified, which would have made this a much stronger study. Under the time constraints of the project this was not an option, due to the length of time required for video analysis. Problems with fish mortality and difficulties in obtaining *A. aquaticus* over the winter and spring months, reduced the number of replicates carried out. Ideally more fish would have been used for each condition, and the means could have been used to compare fish for differences in prey selectivity. It was difficult to obtain larger size ranges of prey throughout the experiments. This is due to the fluctuating size structure of *A. aquaticus* populations throughout the year, a phenomenon documented in a study by Rask and Hiisivouri (1985).

The study could easily be expanded, by examining the foraging behaviour and prey selectivity of ruffe in different densities of *A. aquaticus*. My study on the stomach contents of ruffe demonstrated that even in high densities of *A. aquaticus* a wide range of prey sizes were taken. The selectivity of ruffe may alter under different levels of hunger, a factor that could also be studied. An interesting expansion of the study could be to examine the behaviour of both predators and prey in the presence of different types of shelter. The shelters used in this experiment were artificial, and did not resemble aquatic plants. In contrast comparisons could be made between macrophyte shelters and benthic debris (decaying leaves), which are both natural refuges for *A. aquaticus* within their habitat.

7.3.3 *Ruffe foraging behaviour related to foraging theory*

By limiting the time period the ruffe had to forage in, within the experimental set-up it is not surprising that foraging activity of ruffe was so high in the experiments described in chapter 4. Fish had been trained to feed in the experimental arena, and had previous experience of the time limits on foraging in this situation. Because of this, the high foraging activity could have been a product of them attempting to increase the numbers of prey caught under the time limits of the experiments. Ydenberg and Hurd (1998) describe the foraging model, that foragers will increase their foraging rate when time limits are imposed on foraging period. This may lead to a reduction in foraging efficiency, in an attempt to maximise food rewards in the limited time available. This may account for the high levels of foraging behaviour of ruffe, when foraging in the dark for concealed prey (chapter 4). However the experiment on the foraging behaviour of ruffe on *A. aquaticus* (chapter 5), was also time limited, and was in fact limited to only one hour as opposed to 2, but foraging activity was very low.

There are several reasons that may explain this. The environmental conditions and prey species were very different, and therefore the location and capture of prey was much easier when feeding for relatively active, visible prey in the light than when foraging on concealed prey in the dark. Under the conditions described in chapter 4, fish would be forced to spend a large proportion of their time searching for prey, before even attempting to eat it. In the chapter 5 experiments, fish did not need to seek out prey, as there was a relative abundance of prey that was easy to locate. However, fish still had less time when foraging for *A. aquaticus* than chironomids, so

why did they not eat as many as possible? The low foraging activity meant that foraging and prey capture did not require much energy expenditure, and *A. aquaticus* are a relatively profitable prey species, which are large and a relatively high source of energy. Therefore fish would not need to eat many individuals during the foraging period, providing little energy was used in the feeding process. A greater foraging effort would require a greater prey consumption in order to maximise the net energy gained. In chapter 5 experiments fish had become accustomed to feeding on *A. aquaticus* in the experimental tank, but not under time constraints. Therefore it is possible that the low foraging effort was either because fish were maximising energy gained whilst keeping energy expenditure relatively low, or because they did not know that the foraging period was time limited. In contrast fish in the experiments described in chapter 4 had learned that foraging period was limited, and therefore were trying to feed as much as possible during the time limit.

It is also likely that the foraging conditions influenced the foraging rate. Chironomid larvae are small in relation to *A. aquaticus*, and provided little energy compared to the amount required in order to locate than when concealed under gravel in the dark. (environmental conditions and prey type are know parameters that influence the optimal foraging of species, reviewed in Townsend and Winfield, 1985).
Fish in chapter 5 were foraging much more efficiently than those in chapter 4. This highlights the limitations of foraging for difficult to find prey under suboptimal environmental conditions. The fact that ruffe in chapter 4 were able to forage under such conditions, demonstrates their flexible approach to foraging, which may

contribute to their ability to colonise new habitats, demonstrated by numerous successful invasions.

7.4 Survey of morphological characteristics and parasite presence in a sample of ruffe

The examination of morphological characteristics and parasite infections in ruffe, provided useful information regarding their biology. This corresponded to the existing literature on a variety of aspects relating to ruffe biology and ecology in Loch Lomond, and helped me to put the behavioural work on ruffe into the context of their biology as an invader.

The age range of ruffe in this survey confirmed previous data, that ruffe do not live above 6 years of age. This sample ranged from 0 to 6, with very few individuals above age 4 occurring in the population. The size ranges of ruffe in the sample were smaller than that seen by Adams and Maitland (1998), which may be due to smaller sample size. They observed a tri-modal size distribution in the ruffe population of Loch Lomond. When I examined length frequencies, no such pattern was observed. I did find that females were larger than males, and it is therefore possible that the largest size category in Adams and Maitland (1998) may be large, old females.

This study confirmed that gonadal development occurs in young fish, a fact that had already been described in previous studies (reviewed in Wootten, 1990). Although we did not examine whether fish were sexually mature, clearly differentiated gonads were

found in male fish of age 0 and up, and in female fish from 1 years and up. This supports the evidence that ruffe are highly fecund, which due to their early gonadal development, are able to undergo rapid population expansion. The fact that no correlation was found between GSI and body condition suggests that condition is not compromised by gonad development. The GSI of males was relatively high, when compared to some other species, even though it was significantly smaller that female GSI.

The ruffe sampled had good body condition which was unaffected by parasite burdens. Previous studies (Crowden and Broom, 1980), indicate that *Diplostomum* infections reduce foraging ability. Ruffe appeared unaffected by even high intensity infections, which supports the idea that they utilise their lateral line when foraging. The high levels of D*iplostomum* infections are not unusual in benthic species such as ruffe, due to the levels of exposure to infective cerci in this habitat (Davies and Huffman, 1977).

Very few macroparasites were found in the guts of fish in my sample. Although some individuals were infected with the nematode *Camallanus lacustris*, the rarity of this parasite in the ruffe population can be explained by the ruffe diet. In order to become infected *by C. lacustris*, infected copepods (*Cyclops* sp.) must be eaten. Since copepods are very rare in the diet of ruffe in Loch Lomond, *C. lacustris* is unlikely to be a common parasite of ruffe.

Dorucu (1996) used parasites as indicators of the biology of Arctic charr (*Salvelinus alpinus*). The parasites found in ruffe during this study confirm how clues regarding the habitat use and diet of species can be gained from their parasitic fauna. Considering the importance of *A. aquaticus* in the ruffe diet, I was surprised at the absence of *A. lucii* in the ruffe surveyed. It is possible that this parasite may be rare in the Loch, and that very few infected *A. aquaticus* are present.

7.5 Overall conclusions drawn from this work

This thesis has presented experimental evidence of the foraging ability of ruffe in the absence of visual cues. Ruffe displayed very different foraging tactics when foraging for visible prey in the light. The comparison of these studies highlights the influence of both light intensity and prey species on foraging efficiency in ruffe.

Studies on the behaviour of *A. aquaticus* demonstrate their vulnerability to predation by fish such as ruffe. Female *A. aquaticus* that were carrying broods and pre-copulatory mate-guarding pairs were more sensitive to potential risks, and traded off feeding opportunities in preference for shelter during daylight hours. This proved an effective predator avoidance strategy when predation of *A. aquaticus* by ruffe was studied in the presence of shelters. In the absence of refuges, *A. aquaticus* exploited the reliance of ruffe on prey movements to detect and locate food, by 'freezing' when in close proximity to a fish predator.

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